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# Simultaneous presence of DDT and pyrethroid residues in human breast milk from a malaria endemic area in South Africa

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*The simultaneous presence of DDT and pyrethroid residues in breast milk raises the question of infant exposure and safety.*

**Abstract**

DDT and pyrethroids were determined in 152 breast-milk samples from three towns in KwaZulu-Natal, South Africa, one of which had no need for DDT for malaria control. All compounds were found present in breast milk. Primiparae from one town had the highest mean ΣDDT whole milk levels (238.23 μg/l), and multiparae from the same town had the highest means for permethrin (14.51 μg/l), cyfluthrin (41.74 μg/l), cypermethrin (4.24 μg/l), deltamethrin (8.39 μg/l), and Σpyrethroid (31.5 μg/l), most likely derived from agriculture. The ADI for DDT was only exceeded by infants from one town, but the ADI for pyrethroids was not exceeded. Since the ADI for DDT was recently reduced from 20 to 10 μg/kg/bw, we suggest that this aspect be treated with concern. We therefore raise a concern based on toxicant interactions, due to the presence of four different pyrethroids and DDT. Breastfeeding however, remains safe under prevailing conditions.


*Keywords:* DDT; Pyrethroid; Breast milk; Malaria

## 1. Introduction

In both developing and developed countries, human breast milk remains the best sole nutrient source for infants (especially in the early stages of infancy), even though it could contain pollutants, such as PCBs and DDT (Sonawane, 1995; Pronczuk et al., 2004). The presence of DDT and other organochlorine compounds in human breast milk has been known for quite some time from malaria (Bouwman et al., 1992) and non-malaria areas (Savage et al., 1981). The restriction on or banning of many of these compounds for agriculture in most parts of the world has led to gradual reductions in the levels of organochlorine compounds in breast milk, especially in developed countries such as Canada and Sweden (Van Hove Holdrinet et al., 1977; Bernes, 1998; Shutz et al., 1998).

In Africa where malaria still kills more than a million people each year, the use of pesticides to interrupt the parasite transmission has continued to rely on insecticide treatment of dwellings, and insecticide treated bed nets (Kapp, 2004). The intention of international initiatives such as the Stockholm Convention and the Roll Back Malaria campaign is to reduce the reliance on DDT *per se*, and to use alternative methods, products and strategies. Much of the effort has concentrated on using synthetic pyrethroids as one such alternative, and some countries have successfully done so. One notable exception was the forced reintroduction of DDT in South Africa, after one of the mosquito vectors (that was previously eliminated from South Africa by DDT) returned, but with pyrethroid resistance (Hargreaves et al., 2000). The resulting increase in

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malaria cases and deaths was epidemic – malaria cases soared from 4117 cases in 1995 to 64,622 in 2000.

The Stockholm Convention (Anon, 2004), that was negotiated during this period in Johannesburg, South Africa, took note of this situation and a dedicated section on the conditions of use and production of DDT was negotiated, allowing countries to continue using DDT, but only with strong regulatory and reporting requirements (Anon, 2004). In order to move away from DDT, the Convention urges parties to promote research and development of safe alternative chemical and non-chemical products, methods and strategies relevant to the conditions of those countries, thereby reducing the human and economic burden of the disease. The alternatives, however, should also pose less risk to human health and the environment (Annex B, Part II). Parties are therefore allowed to continue using DDT, but alternatives should be investigated and implemented where possible. Some of the pyrethroids have been promoted, and in many places implemented as alternative indoor residual spray (IRS), or as treatment of bed nets (Goodman et al., 2001). These compounds are generally assumed safer to the environment, and at least benign to human health (Anon, 1998; Ray and Forshaw, 2000; Barlow et al., 2001; WHOPES, 2002). When switching from one chemical to another, and perhaps especially when moving away from DDT, it is inescapable that residues of the former will remain for an extended period (Bouwman et al., 1990a; Yanez et al., 2002). Just as DDT can be taken up by the inhabitants of treated dwellings (Bouwman et al., 1991), so too can humans be exposed to pyrethroids (or other compounds), and potentially excrete both via breast milk. We managed to trace only one published study that has found pyrethroids and organochlorines in the same human milk samples, and that was from Switzerland (malaria control was obviously not the source here) (Zehringer and Herrman, 2001). These authors have found nine pyrethroids (three others were not detected), as well as pyrethrins, in 53 breast-milk samples of mothers living close to Basle, Switzerland.

Malaria control is not the only potential source of human exposure to pesticides. Pyrethroids, organophosphates and carbamates, among others, have now taken over as major crop protection and veterinary chemicals. With increasing development in rural malaria areas, agriculture has changed from mainly subsistence, to a mixture of subsistence, cash crop and commercial farming. This, especially in the case of the northern areas of KwaZulu-Natal (Fig. 1), has meant that more insecticides are now being used. The same areas are still malaria endemic, and many of the homesteads are still subject to annual IRS. Therefore, inhabitants, many of whom are also active in agriculture, may be exposed outdoors in the fields, via food (including animal products) and water, as well as indoors to IRS (Bouwman, 1997). In addition, these compounds can wash into water bodies, and potentially give rise to resistant strains of vectors (Sereda and Meinhardt, 2003).

In developing countries, and especially in the rural areas of these countries, breastfeeding remains (and should be encouraged to remain) a primary source of food to infants. Previous studies done in KwaZulu-Natal, indicated that mothers breastfeed their infants for up to two years. This is a particularly long period, and it can lead to a significant transfer of pollutants to infants (Bouwman et al., 1992). It was found from this and other studies that primiparae mothers had higher concentrations of DDT in their milk (up to double the concentration) than multiparae mothers. The firstborns seem therefore to receive more pollutants via breast milk, when compared with their younger sibs.

This study will investigate the presence of and deliberate on the contribution from possible sources and routes of exposure of pyrethroids and DDT in breast milk of three semi-urban populations that experience various degrees of active malaria control and increased agricultural use of insecticides, some of which are pyrethroids. While previous studies in KwaZulu-Natal only analysed for p,p′-DDE, p,p′-DDT and p,p′-DDT, this study will also look at the o,p′ isomers, due its estrogenic activity (Bitman et al., 1968; Robison et al., 1985; Chen et al., 1997). We will also address some of the implications of these findings, including how it relates to the Stockholm Convention.

## 2. Materials and methods

### 2.1. Study sites

We selected the Ubombo and Ngwavuma districts of the northern parts of the KwaZulu-Natal Province of South Africa. The region lies between Swaziland to the west, Mozambique to the north and the Indian Ocean to the east (Fig. 1). This area is well known due to intensive malaria, biodiversity and agricultural research, and is well characterised in terms of previous DDT investigations on humans and the environment in the 1980s and 1990s. Malaria transmission is endemic but normally low. Malaria transmission is usually between November and December, and April and May. It is a rural area, with few towns – most of the population lives in dispersed homesteads, and all are indigenous and Zulu speaking. Income is derived from agriculture (cotton and sugarcane) and migrant labour. Women and children tend to their gardens and lands, and as paid labourers on small-scale and commercial farms close to the homesteads. Babies and children often accompany their mothers to the fields (Bouwman, 1997). Men are also active in the fields, but many are away, working on farms, mines and in cities.

We collected breast milk from the clinics at the towns of Jozini, Mkuze in Ubombo District, and Kwaliweni in the Ngwavuma District. The first two clinics served people mainly from the low-lying areas where malaria control is endemic, and annual malaria control with IRS is conducted. Currently DDT is used, but between 1995 and 2000 it was replaced with deltamethrin – otherwise DDT was used almost uninterrupted in this area, starting in 1946 (Sharp and le Sueur, 1996). DDT is applied at 2 g/m$^2$, and the pyrethroids at 0.020–0.025 g/m$^2$ (Hargreaves et al., 2000). Assuming an average wall surface area of 32 m$^2$ (4 m × 2 m × 4), each dwelling would receive about 64 g DDT, and 0.64 g of pyrethroid. Dwellings with cement or painted walls are not treated with DDT, but with deltamethrin, because DDT breaks down quickly on cement, and leaves stains on painted surfaces. One of the complex issues faced by malaria control is the tendency of inhabitants to replaster mud walls (for maintenance and appearance) on a yearly basis (Goodman et al., 2001). In 1995, at least 48% of all the dwellings replastered at least some of the walls, compromising the effectiveness of the residually applied insecticide. The inference of this practice is also that it may reduce the uptake of insecticides used as IRS. However, since almost all inhabitants partake in this practice on a semi-regular basis, this factor was deemed as a constant.

The Jozini clinic serves mainly the people from Jozini town itself (which is treated for malaria), as well as people from the Makathini Floodplain associated with the river, downstream from the Pongolopoort Dam. The Mkuze clinic also serves people from malaria areas that need not be treated every year, due to variable presence of vectors. The questionnaires showed however, that all people here were residing in dwellings that were treated once-off for malaria control in 2000 (and not before or since). Also according to the

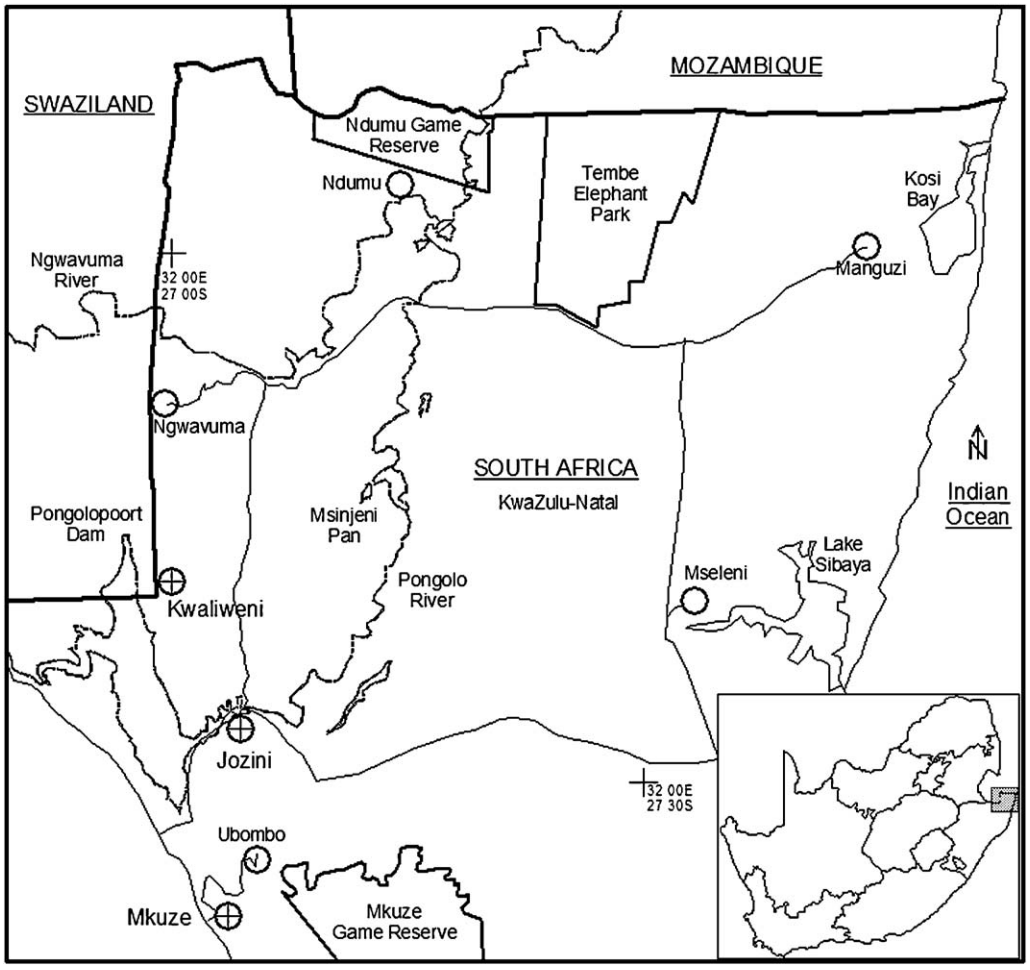


Fig. 1. Map of the northern parts of KwaZulu-Natal, South Africa. The three towns where samples were collected are marked with crossed circles.

questionnaires, all mothers grew up in the areas close to their respective clinics. It is possible however, that legal or illegal immigrants from Mozambique (where DDT is not used) may have participated in the Jozini group, as this town lies directly on the main road from Mozambique (Bouwman et al., 1991). Food items were regulated by availability (drought and poverty playing a large role), rather than preference, and it was difficult to establish any pattern.

The reference site was a clinic (Kwaliweni) in the Lebombo Mountain range between South Africa and Swaziland (Fig. 1). Kwaliweni is the most rural of the three sites, and, although too small to be considered a town, will be referred to as such, for brevity. Malaria does not occur in these higher areas, and the dwellings here have never been treated. The people from and around this clinic do go to the floodplain on occasion, but if they do reside in sprayed dwellings on the floodplain, it is not for very long. They also have access to fish from the Pongolo River floodplain (Msinjeni Pan in the floodplain in particular, where DDT residues have been found in fish (Bouwman et al., 1990a)). Tinned fish was also available from the shops, therefore precluding the floodplain as a sole source of fish. From our inquiries, it was obvious that fish makes up a far lesser proportion of the diet in Kwaliweni than the other two towns. Since fish availability was also erratic, it was not possible to quantify fish intake. Fresh produce (from the Majindi farming area), also reaches Kwaliweni, possibly a vector for insecticides used in crop protection. Drinking water is mainly piped (especially in Jozini), or collected from rivers and streams (mainly in Kwaliweni).

## 2.2. Sample collection

Ethical approval for collection was obtained from Pharma-Ethics and the KwaZulu-Natal Department of Health. A basic questionnaire was completed and prior informed consent obtained from each participant, in Zulu. The samples were collected between April and November of 2002, a period between indoor spraying operations, just after the first resumption of the application of DDT. The mothers therefore experienced five years of no DDT for malaria control (with pyrethroids, mainly deltamethrin instead), followed by one single DDT application, at the time of sampling.

All mothers attending immunisation clinics with well babies were asked to participate and only those that did not want to participate were excluded. According to ethical rules, mothers that did not want to participate were not asked as to the reason why. This was the same procedure used in previous studies (Bouwman et al., 1990b,c), and therefore comparable. Milk was expressed manually into pre-cleaned glass beakers, and 20 ml of milk was collected. Protective measures were taken to prevent sample contamination, as well as collector's exposure to HIV or other pathogens (Fauvel and Ozanne, 1989). The milk was kept on ice, until it could be frozen within two days, and the samples remained frozen until analysis. The timing of the collections were such that first or even second feedings have already been done by the mothers, so that individual lipid levels, that increase from just below 4%, to just above 7% each morning, will have stabilised by then (Hall, 1979), although this time trend was not confirmed in this study.

## 2.3. Analysis

All solvents were analytical grade, and checked for contaminants using process blanks. After defrosting, 10 ml of milk was deprotienised with 30 ml acetone and extracted with *n*-hexane. The extract was cleaned up with sulphuric acid, and the purified extract concentrated and analysed for p,p′-DDT, o,p′-DDT, p,p′-DDE, o,p′-DDE, p,p′-DDD and o,p′-DDD using a GC

fitted with ECD (in-house laboratory method PALSRAM 101). ΣDDT was calculated as the sum of the p,p′ isomers. Percentage DDT was calculated also calculated as the proportion of p,p′-DDT of the ΣDDT.

For pyrethroids, 10 ml of the remaining milk sample was acidified to a pH of about 4 with 1.0 N HCl, and extracted with acetonitrile (50 ml and 25 ml respectively). The extract was cleaned up by extracting with 3 × 15 ml hexane. The cleaned acetonitrile was then evaporated under nitrogen, and the extract cleaned up by passing it through silica gel columns, using diethyl ether and hexane (in-house laboratory method PALSRAM 100). Analysis was done with GC-ECD. We analysed for deltamethrin, cypermethrin, cyfluthrin and permethrin, known to have been used in the area (Goodman et al., 2001; Sereda and Meinhardt, 2003).

Confirmation was done using GC/MS. Quality control was done by a dedicated in-house quality control unit using standard GLP procedures. Although the laboratory is not yet accredited, all procedures are implemented and the laboratory operates according to GLP principles. The laboratory has participated successfully in both the South African organised Inter-laboratory Calibration Exercises (ICEs) and the international FAPAS (Food and Agricultural Proficiency Assessment Scheme), and is well regarded in South Africa.

The limits of quantification (LOQ) for the individual components in whole milk (4% fat) were: p,p′-DDT 0.042 μg/l; o,p′-DDT 0.052 μg/l; p,p′-DDE 0.045 μg/l; o,p′-DDE 0.044 μg/l; p,p′-DDD 0.049 μg/l; o,p′-DDD 0.048 μg/l; permethrin 0.576 μg/l; cyfluthrin 0.181 μg/l; cypermethrin 0.341 μg/l; deltamethrin 0.349 μg/l. However, some values in Table 1 are lower, probably due to variable milk-fat content in the samples.

### 2.4. DDT active ingredient composition

Data on the isomer composition of the DDT used in KwaZulu-Natal was graciously supplied by AVIMA (Pty) Ltd, Johannesburg, South Africa. The percentage p,p′-DDT in the formulation (75% water wettable powder) varied between 72% and 75% of the active ingredient, with the balance made up of o,p′-DDT. Two typical analyses of the technical DDT showed 95.47%/94.27% active, made up of 73.51%/71.84% p,p′-DDT and 21.96%/22.45% o,p-DDT, respectively (analysis by Consulting Chemical Laboratories, Atlasville, South Africa). The other isomers have not been analysed. In 2001, the malaria control programme in KwaZulu-Natal used 15,785 kg DDT (as 75% wwp), 14,508 kg in 2002, and 6483 kg in 2003 (UNEP, 2005).

### 2.5. Statistics

We decided to present data based on both whole milk (wm, Table 1) and milk-fat bases (mf, Table 2). Using both sets of data provided different perspectives of levels, exposure (to infants) and risk (Quinsey et al., 1996), as well as showing some differences when using Anova. Most data in the literature is presented on a milk-fat basis, but ADIs (Allowable Daily Intake) and MRLs (maximum Residue Limit) are calculated or based on whole milk levels, and therefore included here. It has also been shown that milk-fat content increases with the length of lactation, and is therefore correlated with infant age (Quinsey et al., 1996; Nasir et al., 1998). Data were analysed using Prism 4.02 software. Values that could not be quantified were not included in the analysis. ΣDDT was calculated only on the p,p′ isomers, which allowed comparisons with previous work from this area. The levels of many of the pyrethroids were also quite low, and combined with the large proportion of samples having levels below the LOQ, the individual pyrethroids were totalled and analysed as ΣPYR.

Because there were only four mothers comprising the Mkuze primiparae group, the data from this group was only summarised (Tables 1 and 2), but not used in further statistics. One-way analysis of variance was done on log-transformed data, including the ages of the mothers and the milk-fat composition. Although there were some notable outliers in most of the variables (Tables 1 and 2), none were excluded. Analysis was done using one-way ANOVA, with Bonferroni's multiple comparison post-tests for selected pairs of primiparae and multiparae groups from the different towns. Nine logical pairs are possible, but the Mkuze primiparae group was excluded because of low numbers ($n = 4$), and we used only six pairs, as follows: Jozini primiparae with Jozini multiparae and Kwaliweni primiparae; Jozini multiparae with Mkuze multiparae and Kwaliweni multiparae; and Kwaliweni primiparae with Kwaliweni multiparae. Other pairs are possible, but do not present meaningful comparisons. The ANOVA results are presented in Table 3, for both whole-milk and milk-fat based levels. We also investigated different sources of water as a possible determinant. Only in Jozini were there enough mothers using either piped or river water to compare. Un-paired, two-tailed *t*-tests were used to test the means (from log-transformed data) from this group, and the results are presented in Table 4. Only data based on milk fat was used in this comparison.

## 3. Results

### 3.1. Participant profiles

A total of 152 mothers were successfully enrolled in this study. The number of mothers that successfully donated milk at each clinic is presented in Tables 1 and 2. Jozini, being the largest town in the region, had the highest number of participants, with smaller numbers attending the clinics at the other two towns. Ten mothers from Mkuze opted not to participate. All other mothers that were present at the other two clinics did participate. None of the mothers had any accidental or occupational exposure to pesticides, other than working on lands, or through malaria control. Pest control is done on a small scale in home gardens, mainly using a formulated dust containing carbaryl and permethrin, bought in small containers from local shops. In general, the knowledge of the mothers regarding pesticide use and safety was quite limited.

For maternal age there were no significant differences ($P < 0.05$) between the primiparae and multiparae ages between the three towns (Table 3). There were significant differences between the ages of the primiparae and multiparae mothers of Jozini and Kwaliweni – the difference being about seven years in both cases. Based on age, we therefore assumed valid comparisons between pollutant levels in the six selected pairs. The results for infant age (Table 1) reiterate the length of time that mothers from this region breastfeed their infants. The age of the oldest infant still breast-feeding was a tremendous 780 days (more than 2 years old). There were also no significant differences between the levels of milk fat and ages of the infants between any of the groups (Table 3).

### 3.2. The p,p′-DDT isomers

All mothers had detectable levels of p,p′-DDT, except for two from the primiparae group from Mkuze (Tables 1 and 2). Jozini had the highest mean levels followed by Mkuze and Kwaliweni. The primiparae and multiparae from Kwaliweni had mean ΣDDT levels that were very close. All mothers had detectable p,p′-DDE residues (Tables 1 and 2). The mean level for primiparae from Jozini was more than double the mean value for the multiparae from the same town. The mean p,p′-DDE levels for primiparae and multiparae milk from Kwaliweni were relatively close.

Not all mothers had detectable levels of p,p′-DDD (Tables 1 and 2). Surprisingly, hardly more than half of the mothers from Mkuze had detectable levels, while the mothers from Kwaliweni, except for three, had detectable levels. Because

Table 1
Residue values (μg/l) for DDT and pyrethroids based on whole milk from mothers from Jozini, Mkuze and Kwaliweni

| | M Age (years) | Inf Age (days) | PpDDT | ppDDE | PpDDD | ΣDDT | %DDT | opDDT | opDDE | opDDD | Permethrin | Cyfluthrin | Cypermethrin | Deltamethrin | ΣPYR | DDT&PYR (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jozini Primiparae ($n = 33$) | | | | | | | | | | | | | | | | |
| Mean | 19.70 | 148.29 | 70.81 | 165.05 | 2.61 | 238.23 | 33.74 | 11.57 | 1.46 | 1.14 | 10.38 | 3.11 | 1.71 | 1.83 | 8.43 | |
| Median | 19 | 127 | 50.48 | 112.28 | 2.50 | 167.45 | 32.15 | 8.36 | 1.05 | 0.60 | 6.78 | 2.21 | 0.99 | 1.75 | 5.56 | |
| SD | 2.53 | 112.16 | 71.03 | 224.66 | 1.73 | 287.50 | 13.81 | 12.53 | 1.19 | 1.02 | 12.73 | 2.98 | 1.71 | 1.40 | 10.67 | |
| Min | 16 | 14 | 2.25 | 3.62 | 0.39 | 5.87 | 5.81 | 0.26 | 0.15 | 0.14 | 2.18 | 0.42 | 0.14 | 0.18 | 0.18 | |
| Max | 27 | 386 | 291.60 | 1240.40 | 7.92 | 1537.73 | 76.16 | 57.26 | 4.46 | 2.99 | 54.50 | 7.68 | 5.22 | 4.40 | 55.27 | |
| NumPos | | | 33 | 33 | 30 | 33 | 33 | 33 | 15 | 12 | 15 | 5 | 7 | 15 | 25 | 25 |
| %Pos | | | 100 | 100 | 91 | 100 | 100 | 100 | 45 | 36 | 45 | 15 | 21 | 45 | 76 | 76 |
| Jozini Multiparae ($n = 52$) | | | | | | | | | | | | | | | | |
| Mean | 27.62 | 193.87 | 46.94 | 63.82 | 3.62 | 114.38 | 42.65 | 9.18 | 6.96 | 0.43 | 14.51 | 41.74 | 4.24 | 8.39 | 31.50 | |
| Median | 27 | 150 | 45.55 | 50.70 | 2.13 | 105.33 | 40.71 | 7.07 | 0.79 | 0.28 | 12.44 | 1.43 | 0.91 | 2.31 | 12.44 | |
| SD | 5.95 | 163.30 | 28.91 | 49.54 | 5.69 | 71.30 | 14.12 | 8.47 | 15.11 | 0.37 | 16.12 | 126.02 | 9.40 | 20.71 | 109.12 | |
| Min | 18 | 7 | 3.86 | 6.12 | 0.27 | 10.76 | 16.48 | 0.99 | 0.04 | 0.10 | 1.30 | 0.84 | 0.65 | 0.10 | 0.10 | |
| Max | 49 | 681 | 123.51 | 210.05 | 29.62 | 296.05 | 73.98 | 49.01 | 58.63 | 1.37 | 85.12 | 458.56 | 27.50 | 83.08 | 654.26 | |
| NumPos | | | 52 | 52 | 52 | 52 | 52 | 52 | 25 | 25 | 27 | 13 | 8 | 16 | 35 | 35 |
| %Pos | | | 100 | 100 | 100 | 100 | 100 | 100 | 48 | 48 | 52 | 25 | 15 | 31 | 67 | 67 |
| Mkuzi Primiparae ($n = 4$) | | | | | | | | | | | | | | | | |
| Mean | 20.00 | 104.00 | 25.77 | 38.79 | 1.31 | 52.33 | 40.39 | 6.36 | 0.10 | 0.68 | 48.38 | | | | 48.38 | |
| Median | 19 | 104 | 25.77 | 22.45 | 1.31 | 23.25 | 40.39 | 8.26 | 0.10 | 0.68 | 48.38 | | | | 48.38 | |
| SD | 4.08 | 24.49 | 30.82 | 46.38 | 0.42 | 69.16 | 13.68 | 3.33 | 0.00 | 0.00 | | | | | | |
| Min | 17 | 74 | 3.98 | 3.97 | 1.01 | 7.95 | 0.00 | 2.52 | 0.10 | 0.68 | | | | | | |
| Max | 26 | 134 | 47.56 | 106.28 | 1.61 | 154.85 | 50.06 | 8.31 | 0.10 | 0.68 | | | | | | |
| NumPos | | | 2 | 4 | 2 | 4 | 2 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| %Pos | | | 50 | 100 | 50 | 100 | 50 | 75 | 25 | 25 | 25 | 0 | 0 | 0 | 25 | 25 |
| Mkuzi Multiparae ($n = 22$) | | | | | | | | | | | | | | | | |
| Mean | 27.18 | 178.50 | 9.88 | 28.45 | 0.59 | 38.68 | 30.63 | 1.91 | 0.29 | 0.40 | 18.07 | 0.63 | | | 18.20 | |
| Median | 25 | 131 | 6.41 | 12.79 | 0.52 | 21.21 | 28.64 | 1.57 | 0.35 | 0.29 | 19.92 | 0.63 | | | 19.92 | |
| SD | 5.73 | 166.97 | 9.18 | 28.96 | 0.42 | 36.09 | 16.84 | 1.20 | 0.23 | 0.40 | 11.33 | | | | 11.18 | |
| Min | 19 | 7 | 0.54 | 1.24 | 0.09 | 3.52 | 9.22 | 0.41 | 0.03 | 0.01 | 7.02 | | | | 7.32 | |
| Max | 37 | 780 | 32.64 | 99.53 | 1.59 | 124.51 | 78.25 | 4.85 | 0.67 | 1.14 | 34.09 | | | | 34.09 | |
| NumPos | | | 22 | 22 | 13 | 22 | 22 | 19 | 7 | 6 | 5 | 1 | 0 | 0 | 5 | 5 |
| %Pos | | | 100 | 100 | 59 | 100 | 100 | 86 | 32 | 27 | 23 | 5 | 0 | 0 | 23 | 41 |
| Kwaliweni Primiparae ($n = 16$) | | | | | | | | | | | | | | | | |
| Mean | 18.69 | 208.38 | 9.76 | 17.69 | 1.65 | 29.00 | 32.75 | 2.82 | 1.64 | 1.18 | 8.30 | | | | 8.30 | |
| Median | 18 | 183 | 5.80 | 11.01 | 1.30 | 19.53 | 30.00 | 2.05 | 1.02 | 0.69 | 4.99 | | | | 4.99 | |
| SD | 2.12 | 168.01 | 10.40 | 22.87 | 1.42 | 31.95 | 13.69 | 2.04 | 1.50 | 1.26 | 7.39 | | | | 7.39 | |
| Min | 16 | 14 | 2.16 | 4.08 | 0.13 | 7.57 | 17.36 | 0.52 | 0.30 | 0.14 | 1.45 | | | | 1.45 | |
| Max | 23 | 614 | 39.99 | 99.73 | 5.19 | 141.79 | 60.76 | 7.06 | 3.51 | 4.58 | 25.60 | | | | 25.60 | |
| NumPos | | | 16 | 16 | 15 | 16 | 16 | 16 | 8 | 11 | 10 | 0 | 0 | 0 | 10 | 10 |
| %Pos | | | 100 | 100 | 94 | 100 | 100 | 100 | 50 | 69 | 63 | 0 | 0 | 0 | 63 | 63 |
| Kwaliweni Multiparae ($n = 25$) | | | | | | | | | | | | | | | | |
| Mean | 26.60 | 199.04 | 7.41 | 17.51 | 1.92 | 26.69 | 27.99 | 3.36 | 1.90 | 1.23 | 7.34 | 2.53 | | | 7.51 | |
| Median | 27 | 180 | 5.40 | 16.37 | 1.30 | 21.98 | 24.06 | 2.09 | 0.91 | 0.61 | 7.50 | 2.53 | | | 7.50 | |
| SD | 5.86 | 150.23 | 7.32 | 12.65 | 1.90 | 17.75 | 12.82 | 2.98 | 2.49 | 1.40 | 3.46 | | | | | |
| Min | 19 | 7 | 1.23 | 0.63 | 0.26 | 2.12 | 12.71 | 0.52 | 0.03 | 0.14 | 1.86 | | | | | |
| Max | 40 | 524 | 31.11 | 49.40 | 8.17 | 66.76 | 58.02 | 10.72 | 8.23 | 5.94 | 15.47 | | | | | |
| NumPos | | | 25 | 25 | 23 | 25 | 25 | 25 | 17 | 25 | 15 | 1 | 0 | 0 | 15 | 15 |
| %Pos | | | 100 | 100 | 92 | 100 | 100 | 100 | 68 | 100 | 60 | 4 | 0 | 0 | 60 | 60 |

Table 2
Residue values (μg/kg) for DDT and pyrethroids based on milk fat from mothers from Jozini, Mkuze and Kwaliweni

| | M Age (years) | Milk fat (%) | ppDDT | ppDDE | ppDDD | ΣDDT | %DDT | OpDDT | opDDE | opDDD | Permethrin | Cyfluthrin | Cypermethrin | Deltamethrin | ΣPYR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jozini Primipare ($n = 33$) | | | | | | | | | | | | | | | |
| Mean | 19.70 | 4.32 | 1989.90 | 4170.08 | 85.29 | 6237.52 | 33.74 | 340.14 | 42.14 | 24.80 | 273.52 | 128.41 | 33.34 | 59.87 | 235.05 |
| Median | 19 | 3 | 1336.67 | 3155.00 | 56.46 | 4980.00 | 32.15 | 200.71 | 36.30 | 20.00 | 187.83 | 81.85 | 26.33 | 37.44 | 197.78 |
| SD | 2.53 | 2.90 | 2038.62 | 3828.51 | 86.27 | 5600.73 | 13.81 | 408.80 | 32.16 | 18.63 | 246.67 | 149.12 | 28.08 | 67.46 | 220.45 |
| Min | 16 | 1.1 | 79.86 | 164.55 | 12.96 | 266.82 | 5.81 | 11.82 | 4.05 | 5.49 | 50.70 | 5.38 | 3.50 | 4.05 | 7.50 |
| Max | 27 | 14.5 | 9685.93 | 13,782.22 | 440.00 | 22,241.11 | 76.16 | 2120.74 | 123.89 | 61.11 | 1009.26 | 364.17 | 81.33 | 200.00 | 1023.52 |
| NumPos | | | 33 | 33 | 30 | 33 | 33 | 33 | 15 | 12 | 15 | 5 | 7 | 15 | 25 |
| %Pos | | | 100 | 100 | 91 | 100 | 100 | 100 | 45 | 36 | 45 | 15 | 21 | 45 | 76 |
| Jozini Multipare ($n = 52$) | | | | | | | | | | | | | | | |
| Mean | 27.62 | 3.65 | 1395.53 | 1865.69 | 97.03 | 3358.24 | 42.65 | 261.17 | 153.63 | 13.87 | 400.41 | 1307.84 | 126.96 | 257.96 | 941.60 |
| Median | 27 | 3.45 | 1255.42 | 1419.83 | 60.56 | 2737.59 | 40.71 | 223.19 | 21.11 | 8.00 | 251.85 | 44.33 | 27.50 | 46.07 | 287.50 |
| SD | 5.95 | 1.48 | 913.10 | 1509.96 | 142.27 | 2221.04 | 14.12 | 189.07 | 300.04 | 13.99 | 497.67 | 3836.81 | 285.67 | 648.33 | 3321.11 |
| Min | 18 | 1.2 | 140.30 | 115.77 | 11.46 | 446.67 | 16.48 | 13.94 | 1.82 | 2.86 | 28.26 | 15.56 | 11.02 | 5.00 | 5.00 |
| Max | 49 | 8.4 | 4174.80 | 6642.22 | 955.48 | 10,107.78 | 73.98 | 1065.43 | 841.94 | 69.17 | 2579.39 | 13,895.76 | 833.33 | 2517.58 | 19,826.06 |
| NumPos | | | 52 | 52 | 52 | 52 | 52 | 52 | 25 | 25 | 27 | 13 | 8 | 16 | 35 |
| %Pos | | | 100 | 100 | 100 | 100 | 100 | 100 | 48 | 48 | 52 | 25 | 15 | 31 | 67 |
| Mkuzi Primipare ($n = 4$) | | | | | | | | | | | | | | | |
| Mean | 20 | 3.03 | 1640.61 | 2116.26 | 56.03 | 2964.58 | 40.39 | 283.83 | 2.78 | 18.89 | 1343.89 | | | | 1343.89 |
| Median | 18.5 | 3.50 | 1640.61 | 634.72 | 56.03 | 657.08 | 40.39 | 230.83 | 2.78 | 18.89 | 1343.89 | | | | 1343.89 |
| SD | 4.08 | 1.02 | 2163.83 | 3327.22 | 15.99 | 4914.45 | 13.68 | 244.68 | 0.00 | 0.00 | | | | | |
| Min | 17 | 1.50 | 110.56 | 110.28 | 44.72 | 220.83 | 0.00 | 70.00 | 2.78 | 18.89 | | | | | |
| Max | 26 | 3.60 | 3170.67 | 7085.33 | 67.33 | 10,323.33 | 50.06 | 550.67 | 2.78 | 18.89 | | | | | |
| NumPos | | | 2 | 4 | 2 | 4 | 2 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| %Pos | | | 50 | 100 | 50 | 100 | 50 | 75 | 25 | 25 | 25 | 0 | 0 | 0 | 25 |
| Mkuzi Multipare ($n = 22$) | | | | | | | | | | | | | | | |
| Mean | 27.18 | 3.97 | 306.72 | 832.82 | 16.42 | 1149.24 | 30.63 | 57.69 | 10.06 | 12.00 | 493.67 | 26.25 | | | 498.92 |
| Median | 25 | 4.00 | 162.60 | 405.42 | 8.57 | 525.96 | 28.64 | 43.90 | 8.43 | 7.04 | 498.00 | 26.25 | | | 498.00 |
| SD | 5.73 | 2.15 | 354.21 | 972.77 | 14.53 | 1241.48 | 16.84 | 51.82 | 11.13 | 14.36 | 270.14 | 0.00 | | | 265.47 |
| Min | 19 | 2.00 | 24.41 | 30.24 | 4.50 | 105.97 | 9.22 | 12.81 | 0.75 | 0.21 | 178.54 | 0.00 | | | 178.54 |
| Max | 37 | 12.00 | 1490.00 | 3714.00 | 54.83 | 4458.00 | 78.25 | 242.50 | 33.50 | 39.31 | 852.25 | 0.00 | | | 852.25 |
| NumPos | | | 22 | 22 | 13 | 22 | 22 | 19 | 7 | 6 | 5 | 1 | 0 | 0 | 5 |
| %Pos | | | 100 | 100 | 59 | 100 | 100 | 86 | 32 | 27 | 23 | 5 | 0 | 0 | 23 |
| Kwaliweni Primipare ($n = 16$) | | | | | | | | | | | | | | | |
| Mean | 18.69 | 4.66 | 272.43 | 411.06 | 47.46 | 727.99 | 32.75 | 80.54 | 61.80 | 44.21 | 186.02 | | | | 186.02 |
| Median | 18 | 4.15 | 135.79 | 230.04 | 31.36 | 415.05 | 30.00 | 44.68 | 24.25 | 17.25 | 148.68 | | | | 148.68 |
| SD | 2.12 | 2.07 | 343.35 | 385.94 | 67.18 | 668.72 | 13.69 | 83.22 | 83.50 | 70.38 | 150.29 | | | | 150.29 |
| Min | 16 | 1.70 | 40.75 | 82.05 | 2.45 | 138.59 | 17.36 | 9.81 | 5.56 | 2.00 | 24.87 | | | | 24.87 |
| Max | 23 | 9.50 | 1305.88 | 1511.06 | 273.16 | 2149.41 | 60.76 | 275.26 | 206.47 | 241.05 | 465.45 | | | | 465.45 |
| NumPos | | | 16 | 16 | 15 | 16 | 16 | 16 | 8 | 11 | 10 | 0 | 0 | 0 | 10 |
| %Pos | | | 100 | 100 | 94 | 100 | 100 | 100 | 50 | 69 | 63 | 0 | 0 | 0 | 63 |
| Kwaliweni Multipare ($n = 25$) | | | | | | | | | | | | | | | |
| Mean | 26.60 | 3.40 | 247.34 | 567.52 | 61.03 | 871.01 | 27.99 | 113.74 | 65.66 | 37.34 | 223.56 | 66.58 | | | 228.00 |
| Median | 27 | 3.10 | 187.83 | 554.42 | 35.45 | 797.22 | 24.06 | 74.78 | 19.42 | 24.40 | 211.07 | 66.58 | | | 211.07 |
| SD | 5.86 | 1.59 | 274.62 | 408.16 | 72.48 | 596.16 | 12.82 | 109.66 | 97.47 | 38.87 | 119.56 | 0.00 | | | 127.81 |
| Min | 19 | 1.10 | 44.74 | 33.16 | 13.68 | 111.58 | 12.71 | 22.41 | 2.50 | 4.83 | 40.43 | 66.58 | | | 40.43 |
| Max | 40 | 7.90 | 1244.40 | 1660.00 | 291.79 | 2168.00 | 58.02 | 400.00 | 283.79 | 148.57 | 411.30 | 66.58 | | | 473.68 |
| NumPos | | | 25 | 25 | 23 | 25 | 25 | 25 | 17 | 25 | 15 | 1 | 0 | 0 | 15 |
| %Pos | | | 100 | 100 | 92 | 100 | 100 | 100 | 68 | 100 | 60 | 4 | 0 | 0 | 60 |

Table 3
Results of ANOVA and Bonferroni post-tests for pairs of comparisons for both whole milk and milk fat based residue means

| | M Age | Inf Age | ppDDT | ppDDE | ppDDD | ΣDDT | %DDT | opDDT | opDDE | opDDD | ΣPYR | %mf |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whole milk | | | | | | | | | | | | |
| One way ANOVA | $P < 0.0001$ | $P = 0.9425$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P = 0.0002$ | $P < 0.0001$ | $P = 0.0919$ | $P = 0.0027$ | $P = 0.5446$ | |
| Joz Prim vs Joz Multi | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | |
| Joz Prim vs Kwal Prim | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P > 0.05$ | $P < 0.001$ | $P > 0.05$ | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | |
| Joz Multi vs Mkuzi Multi | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ | $P < 0.001$ | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ | |
| Joz Multi vs Kwal Multi | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | |
| Mkuzi Multi vs Kwal Multi | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.01$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | |
| Kwal Prim vs Kwal Multi | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | |
| Milk fat | | | | | | | | | | | | |
| One way ANOVA | $P < 0.0001$ | $P = 0.9425$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P = 0.0002$ | $P < 0.0001$ | $P = 0.1166$ | $P = 0.0048$ | $P = 0.4836$ | $P = 0.3210$ |
| Joz Prim vs Joz Multi | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ |
| Joz Prim vs Kwal Prim | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.05$ | $P < 0.001$ | $P > 0.05$ | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ |
| Joz Multi vs Mkuzi Multi | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ |
| Joz Multi vs Kwal Multi | $P > 0.05$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P > 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ |
| Mkuzi Multi vs Kwal Multi | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.01$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P < 0.05$ | $P > 0.05$ | $P > 0.05$ |
| Kwal Prim vs Kwal Multi | $P < 0.001$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ | $P > 0.05$ |

Table 4
Results of unpaired two-tailed *t*-tests on milk fat based residue means for breast milk of mothers from Jozini depending either on river or piped water

| | M Age (years) | Inf Age (days) | milk fat (%) | ppDDT | ppDDE | ppDDD | ΣDDT | %DDT | opDDT | opDDE | opDDD | Permethrin | Deltamethrin | ΣPYR (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| River water $n = 23$ | | | | | | | | | | | | | | |
| Mean | 22.70 | 190.95 | 2.98 | 2220.07 | 3820.74 | 101.54 | 6137.94 | 38.84 | 376.42 | 46.77 | 16.99 | 515.39 | 376.36 | 509.57 |
| Median | 21.00 | 127.00 | 3.00 | 1877.74 | 3026.33 | 88.34 | 5350.30 | 36.76 | 322.33 | 33.15 | 11.67 | 158.25 | 64.29 | 120.54 |
| SD | 5.18 | 178.93 | 1.07 | 1382.58 | 2990.43 | 50.82 | 4090.89 | 11.59 | 242.27 | 45.78 | 14.30 | 851.68 | 867.88 | 1331.60 |
| Min | 17 | 7 | 1.20 | 412.92 | 657.14 | 35.45 | 1185.42 | 19.57 | 79.17 | 3.41 | 4.12 | 73.33 | 5.00 | 5.00 |
| Max | 31 | 607 | 6.00 | 5487.67 | 11,027.33 | 198.06 | 16,614.67 | 64.84 | 907.00 | 136.13 | 46.33 | 2579.39 | 2517.58 | 5096.97 |
| NumPos | | | 23 | 23 | 23 | 22 | 23 | 23 | 23 | 10 | 11 | 8 | 8 | 14 |
| %Pos | | | 100 | 100 | 100 | 96 | 100 | 100 | 100 | 43 | 48 | 35 | 35 | 61 |
| Piped water $n = 51$ | | | | | | | | | | | | | | |
| Mean | 25.39 | 166.67 | 3.93 | 1323.55 | 2066.68 | 94.46 | 3418.15 | 40.52 | 235.19 | 164.26 | 15.52 | 329.73 | 108.05 | 283.83 |
| Median | 24.00 | 134.00 | 3.60 | 1223.13 | 1278.26 | 52.73 | 2670.48 | 36.98 | 167.41 | 24.10 | 9.26 | 252.46 | 37.67 | 187.00 |
| SD | 6.84 | 137.37 | 1.94 | 1056.91 | 2520.67 | 153.91 | 3324.86 | 16.44 | 199.67 | 310.49 | 15.38 | 269.12 | 233.94 | 307.82 |
| Min | 16 | 14 | 1.10 | 102.27 | 115.77 | 11.46 | 266.82 | 5.81 | 11.82 | 1.82 | 2.86 | 28.26 | 4.05 | 4.05 |
| Max | 49 | 681 | 9.00 | 4555.56 | 13,782.22 | 955.48 | 17,085.89 | 76.16 | 1065.43 | 841.94 | 69.17 | 1009.26 | 995.42 | 1247.27 |
| NumPos | | | 51 | 51 | 51 | 50 | 51 | 51 | 51 | 23 | 21 | 28 | 17 | 39 |
| %Pos | | | 98 | 98 | 98 | 96 | 98 | 98 | 98 | 44 | 40 | 54 | 33 | 75 |
| *T*-Test *P* value | 0.0836 | 0.5639 | 0.0751 | 0.0373 | 0.002 | 0.0315 | 0.0011 | 0.9717 | 0.0041 | 0.9342 | 0.7454 | 0.9611 | 0.5613 | 0.4827 |

of the low numbers of participants from Mkuze, and the low percentage of samples with detectable p,p′-DDD residues in all mothers from Mkuze, the interpretative confidence of its p,p′-DDD data is probably compromised.

As would be expected from the ubiquitous presence of p,p′-DDE, all mothers were also positive for the sum (ΣDDT) that includes this metabolite (Tables 1 and 2). The Jozini primiparae had the highest mean level, with the multiparae from the same town, having the second highest mean, even though this was about half the mean primiparae level (Tables 1 and 2).

The %DDT (Tables 1 and 2) was greatest in the Jozini multiparae (42.65%), followed by the Mkuze primiparae, although these had low numbers. In Kwaliweni and Mkuze, the multiparae had a higher %DDT than the primiparae from the same town, but it was the other way round for Jozini.

The Anova (Table 3) showed highly significant differences ($P < 0.001$) for p,p′-DDT, p,p′-DDE, p,p′-DDD and ΣDDT for both the whole-milk and milk-fat calculated data, as well as the %DDT (percentage calculations are independent of the method of calculation − whole milk or milk fat).

### 3.3. The o,p′ DDT isomers

In general, the o,p′ levels for DDT, DDE and DDD were, as expected, much lower than their p,p′ counterparts (Tables 1 and 2). The Jozini primiparae and multiparae once again had the highest mean levels of o,p′-DDT, even though the medians were close together (Tables 1 and 2). Although the percentage of mothers with detectable levels of o,p′-DDE was low, it was remarkable that this percentage was the highest in Kwaliweni. The Jozini multiparae, however, had the highest mean o,p′-DDE level. For o,p′-DDD, the percentage of mothers with detectable levels was again higher in Kwaliweni, and they also had the highest mean levels, followed by Jozini (Tables 1 and 2).

The Anova showed the same significant differences for o,p′-DDT, as for p,p′-DDT and p,p′-DDE for both whole-milk and milk-fat based data (Table 3). For whole-milk data, however, the Jozini multiparae had significantly higher mean levels of o,p′-DDE when compared with the mean for the Mkuze multiparae. The Kwaliweni multiparae had significantly higher mean o,p′-DDD levels than their counterparts in Jozini and Mkuze (Table 3).

### 3.4. Pyrethroids

Permethrin was the compound most often found at quantifiable levels in the breast-milk samples collected, followed by cyfluthrin and deltamethrin (Tables 1 and 2). Cypermethrin and deltamethrin were not present in quantifiable levels in Mkuze and Kwaliweni. It also appears that Mkuze had the least number of samples with quantifiable levels of all pyrethroids. Of the 152 samples, 101 or 66% had detectable quantities of pyrethroids. The highest levels was from one mother from the Jozini multiparae group. Because of the exceedingly high levels, these are listed here: permethrin − 85.12 μg/l wm; 2579 μg/kg mf, 458.56 μg/l wm; 13,895.76 μg/kg mf cyfluthrin, 27.50 μg/l wm; 833.33 μg/kg mf cypremethrin, and

83.08 μg/l wm; 2517.59 μg/kg mf deltamethrin, for a ΣPYR level of 654.26 μg/l wm; 19,826.06 μg/kg mf. Her ΣDDT level was 176.65 μg/l wm; 5350.30 μg/kg mf.

As was the case for the o,p′ compounds, the variability and low percentage of samples with quantifiable levels of pyrethroids were such that we also summed all pyrethroids (ΣPYR; Tables 1 and 2) for comparisons. With the large variation in the data, it is also perhaps not surprising that the Anova did not show any significant difference (Table 3).

The percentage of samples that had quantifiable levels of any pyrethroid and any DDT compound (DDT&PYR) was the highest in Jozini (76% primiparae and 67% multiparae), followed by Kwaliweni (63% primiparae and 60% multiparae; Table 1).

### 3.5. Water sources

The participants from the three clinics utilised different water sources for domestic use – drinking, cooking and washing. In Jozini, 23 mothers (27%) obtained their water from rivers and streams, 9 (10%) from bore holes, 2 (2.4%) used spring water, and 51 (60%) used piped water. Eight mothers (36.4%) from Mkuze relied mainly on rivers, two each (9.1%) relied on spring and bore-hole water, and 12 (45.4%) used piped water. Kwaliweni, a more isolated town, mainly relied on river water (32 mothers, 78%), with eight mothers (19.5%) using bore-hole water, and one (2.4%) using piped water. Since only Jozini had reasonable numbers of mothers using either piped or river water, these two groups were compared using un-paired two-tailed *t*-tests on log-transformed data (Table 4). There were no significant differences between maternal age, infant age and percentage milk fat between the two groups, but the river group were younger, and had a lower percentage milk fat. It should also be kept in mind that for some parameters, such as o,p′-DDE, o,p′-DDD, and the pyrethroids, only a few from the river water group had detectable levels.

Water source as a factor indicated no differences (Table 4) for pyrethroids, but, unexpectedly, it did indicate significant differences for some of the DDT parameters. For p,p′-DDT, p,p′-DDE, o,p′-DDT and ΣDDT, the differences in mean levels were significant, and always lower in the mothers depending on piped water. Even more remarkable was that the mothers using piped water had a remarkably consistent 54–66% lower mean level for these three parameters. For p,p′-DDD a somewhat different picture emerged. Although the means were statistically different, the means were almost the same. The %DDT, however, was not significantly different between these two groups, indicating that the proportions that they measured remained about the same. There were also no significant differences for the three pyrethroid parameters, although those using piped water generally had higher levels.

## 4. Discussion

### 4.1. Comparison with previous studies

Relevant to the objectives of this study, three major changes have taken place since the previous breast milk surveys done in 1987–88 (Bouwman et al., 1990b,c, 1992); the much more extensive use of insecticides in agriculture, the interim period of pyrethroid use for malaria control, and the first year of resumption of DDT application (when the samples for this study were collected), following the failure of pyrethroids. The effects of all three of these changes can be identified, with some constraints, when comparing the data from the '80s, with the present levels.

Since the objectives and design of the Mseleni study differed from the present study, comparisons of levels of DDT in breast milk will be indicative rather than conclusive. The Mseleni study was a repeated cross-sectional study on mothers attending regular well-baby clinics to investigate the changes in DDT levels associated with DDT application. Due to the small sample size of the individual surveys in 1987, only crude comparisons on pooled data from both studies would be relevant (Table 5). It is immediately apparent that the levels in the present study were lower than from 14 years before. ΣDDT was less by about 60%, for p,p′-DDT, p,p′-DDD it was less by 75–80%, and 66% for p,p′-DDE. Interestingly, %DDT was only 14% less.

Provisionally assuming that the two groups are comparable, other than Jozini having had a five year interruption in DDT application followed by one year of DDT, two qualified deductions can be made. (1) The single application of DDT in Jozini has not been enough to allow milk levels (p,p′-DDT and p,p′-DDE) to reach those associated with uninterrupted DDT application. Although DDT depuration (over five years of no DDT application, and although DDT will still be present in the general environment and taken up (Bouwman et al., 1990b)) and assimilation (following the first application after resumption of DDT) rates are not known, it is obvious that it will take some time for the previous (assumed) levels to be reached again. (2) The difference in %DDT is, however, much closer between the two groups, and indicates that in Jozini, DDT is being assimilated much faster than depuration, but that the rate of assimilation has not yet reached a level associated from a regular yearly application, as was the exposure profile of the Mseleni mothers. The above however, needs to be interpreted with caution, and requires further investigation.

Comparisons with DDT levels in breast milk from other countries shows that the levels found in this study are within the same order of magnitude or less. Mean ΣDDT (including o,p′-DDT) in 40 mothers from Harare was 6000 μg/kg mf, ranging between 590 μg/kg mf and 55,500 μg/kg mf (Chikuni et al., 1991). It was not clear whether these mothers were

Table 5
General comparison of results from Mseleni (1987) and Jozini (2001) residue levels based on milk fat

| | Jozini mf | Mseleni mf | Proportion | Jozini wm | Mseleni wm | Proportion |
|---|---|---|---|---|---|---|
| DDT | 1626 | 6770 | 0.24 | 56 | 242 | 0.23 |
| DDE | 2760 | 8650 | 0.32 | 103 | 316 | 0.33 |
| DDD | 93 | 400 | 0.23 | 3 | 16 | 0.19 |
| ΣDDT | 4476 | 15,830 | 0.28 | 163 | 575 | 0.28 |
| %DDT | 39.19 | 45.71 | 0.86 | 39.19 | 45.71 | 0.86 |

exposed to DDT via malaria control. From Delhi, India, about 25 mothers had a mean ΣDDT level of 1270 μg/kg mf, ranging between 330 μg/kg mf and 4110 μg/kg mf (Nair et al., 1996). These mothers were apparently not exposed to DDT from malaria control. A large sample ($n = 411$) from Jordan had a mean ΣDDT level of 9700 μg/kg mf; 330 μg/l wm, with a maximum of 67,080 μg/kg mf ΣDDT (Nasir et al., 1998). The sources of DDT were from ''medical hygiene'' and agriculture. From Uganda, 60 samples from Kampala had a mean ΣDDT level of 3970 μg/kg mf, ranging from 880 μg/kg mf, to 18,520 μg/kg mf (Ejobi et al., 1996). Although it was mentioned that DDT was used for mosquito control in Uganda at the time of sampling, the route of exposure was not stated.

We could trace only one study reporting on pyrethroids in breast milk (Zehringer and Herrman, 2001). Three of the four pyrethroids we analysed were also present in Swiss samples collected in 1998/99. The mean and maxima for the following were reported, and can be compared with the data in Table 2: permethrin 74 μg/kg mf and 152 μg/kg mf; cyfluthrin 23 μg/kg mf and 147 μg/kg mf; 36 μg/kg mf and 156 μg/kg mf; deltamethrin was not detected, and the minima for all pyrethroids were below detection limit. The ΣPYR in the Swiss study was <15 μg/kg mf to 450 μg/kg mf, which compared with 5 μg/kg mf and 19,826.06 μg/kg for the Jozini multiparae. The means from Jozini were therefore one order of magnitude higher, and the maxima two orders of magnitude higher.

### 4.2. General comparisons between groups

There were no significant differences in infant age and milk fat between any of the groups that we compared. The maternal ages differed significantly between the primiparae and multiparae mothers of each town – the means differ by about seven years in each town. We can therefore safely compare the other variables, but keeping in mind that we had few primiparae mothers from Mkuze.

The participants from Jozini have had lifelong exposure to DDT via annual application, interrupted for five years, and then resumed for one year, prior to sampling. The mothers from Mkuze had a more variable exposure, as they may not have resided in dwellings that were treated annually, and the mothers from Kwaliweni had indicated no residence in any treated dwelling. The DDT based results corroborates this. The higher mean levels of p,p′-DDT, and the higher mean %DDT in the Jozini samples show recent exposure to the parent compound, and it can safely be assumed that this is derived largely from IRS. The presumably variable application experienced in Mkuze had a corresponding wide range of values in most respects, and the Kwaliweni mothers had even lower levels, but numbers of samples with detectable amounts almost similar to Jozini.

### 4.3. The p,p′-DDT isomers

For both p,p′-DDT in whole milk and milk fat, the Jozini mothers had significantly higher mean levels when compared with the mothers from Kwaliweni, the reference town for this study (Tables 1–3). The p,p′-DDT levels were seven times higher in Jozini primiparae, and the p,p′-DDE levels were four times higher. The greater difference for p,p-DDT can certainly be ascribed to the active application of DDT as IRS in the dwellings of the Jozini mothers. The lesser difference for p,p′-DDE is probably due to the more ubiquitous and stable nature of DDE, and that the first assimilation of p,p′-DDT by the Jozini mothers after five years, has not yet been converted to p,p′-DDE.

p,p′-DDT was 4.5 times higher in Jozini than in Mkuze, and 5.6 greater than in Kwaliweni (Tables 1–3). For the p,p′-DDE the respective differences were 10.1 and 3.3 times. Although the difference was not significant, the Jozini primiparae also had p,p′-DDE levels more than double the multiparae from the same town, while the comparable difference was much less for p,p′-DDT.

A similar picture emerges when considering mean ΣDDT (Tables 2 and 3). The Jozini primiparae had 8.6 times higher ΣDDT than their counterparts in Kwaliweni, and the Jozini multiparae had 2.9 and 3.8 times more ΣDDT than those from Mkuze and Kwaliweni respectively (based on milk fat). However, it is noteworthy that the levels in the primiparae from Jozini were so much greater, when compared with any other multiparae groups. The levels were almost double the next highest level of the multiparae from the same town. It is therefore important to recognise, not only from this study, but also from others (Rogan et al., 1986; Bouwman et al., 1992; Ejobi et al., 1998; Minh et al., 2004), that the first-born infants from mothers with elevated levels of DDT in their breast milk (and even some other pollutants), compose a subset of the population that have a higher risk, due to these elevated levels. It also needs to be mentioned that at low levels of pollution a reverse effect of parity on levels has been observed (Mussalo-Rauhamaa et al., 1988; Albers et al., 1996; Czaja et al., 1997).

Although the ΣDDT is 8.3, and p,p′-DDT 7 times higher in the Jozini primiparae than in the Kwaliweni primiparae, there is hardly any difference in the mean %DDT between the two groups (Tables 1–3). The large and significant difference between the primiparae and multiparae from within Jozini is therefore surprising. Jozini was also the only town where the %DDT was higher in the multiparae than in the primiparae; the situation was reversed (although not significantly) in the other two towns. The multiparae from Kwaliweni, although having a higher mean ΣDDT than the primiparae from the same town, had a smaller %DDT; in fact the lowest %DDT from any group. This value was also significantly different from the multiparae from Jozini.

A pattern emerges where the differences between these two groups (the multiparae from Jozini and Kwaliweni) for p,p′-DDT, p,p′-DDE, ΣDDT, %DDT, and later on also for o,p′-DDD and o,p′-DDT, indicate differences on three possible levels; exposure established difference in DDT application, the major routes of uptake (such as food, water and air), and in the pharmacokinetics and pharmacodynamics between the different isomers (Cai et al., 1995; Kitamura et al., 2002).

p,p′-DDD has always been somewhat of an enigma in studies like these; often measured, often included as part of ΣDDT and %DDT parameters, but little discussed. Its levels are generally low, and it is therefore not detected in all samples. In this study, it was not detected in 17 samples. Mkuze was also the town with the lowest number of samples with detectable p,p′-DDD levels (15 out of 26), while for the other towns it was more than 90%. The multiparae from both Jozini and Kwaliweni also had higher mean levels when compared with the primiparae. The multiparae from Mkuze had significantly lower levels than their counterparts from the other two towns. The pattern of p,p′-DDD levels is inconsistent with those from the other p,p′ isomers, providing little opportunity for explanation. One remote possibility is that some fish species seem to have elevated levels of p,p′-DDD from certain pans near Ndumu (Fig. 1) (Bouwman et al., 1990a), but that would require both Jozini and Kwaliweni mothers to have comparable access to fish from these parts as a regular part of their diet, which is unlikely. Another possibility might be the differences in major sources of domestic water. In Jozini, people mainly derived their water from piped reticulation, while river water was the major source for Kwaliweni mothers. This aspect will be discussed further on.

### 4.4. The o,p′-DDT isomers

Since o,p′-DDT is normally found as part of the technical DDT applied, it comes as no surprise that this isomer follows the same pattern as for p,p′-DDT and p,p′-DDE, and therefore also for ΣDDT (Tables 1–3). The differences in mean levels (those that are significant; Table 3) are not as pronounced, however; the Jozini primiparae had only 4.5 times as much o,p′-DDT as the Kwaliweni primiparae (7 times and 10.1 times for p,p′-DDT and p,p′-DDE respectively), and the Mkuze multiparae had 4.5 times less and 2.0 times more o,p′-DDT than the respective groups from Jozini and Kwaliweni (4.5 and 5.6 times for p,p′-DDT, and 2.2 and 3.2 times for p,p′-DDE for the differences between the multiparae from Mkuze and Jozini, and between the same for Mkuze and Kwaliweni, respectively). The other notable finding was that only four mothers from the entire group had no detectable o,p′-DDT levels (Tables 1 and 2). Again, the presence of o,p′-DDT in breast milk from Kwaliweni, indicates either some sort of exposure to DDT applied close by, although at much lower levels, or through water or food – the source and route of exposure is not known.

The mean levels for o,p′-DDE were lower than for o,p′-DDT (Tables 1 and 2). The multiparae from Jozini had a significant 24-times higher mean level than the multiparae from Mkuze in whole milk (Table 2), but in whole milk it was not significant at 15 times (Table 3). However, the low percentage of samples with detectable levels indicates caution regarding any interpretations. Again, it seems that the multiparae from each town had levels higher (and also had slightly higher percentage of samples with detectable levels), than their respective primiparae, regarding o,p′-DDE. In all of the positive cases from the Jozini primiparae, the o,p′-DDE levels were less by at least one order of magnitude. How and why these differences have occurred is not known.

The percentage mothers with detectable levels of o,p′-DDD was greater in all groups, when compared with o,p′-DDE (Tables 1 and 2). Significant differences were again seen between the multiparae from Kwaliweni, with the multiparae from Jozini and Mkuze respectively (Table 3).

Apart from being confusing, these differences in o,p′ isomer parameters (or lack of) show that the relative contributions from different o,p′ isomers differ between towns, parity status of the mothers, and that the basis for calculation (whole milk or milk fat) need to be taken into account. Comparing the minor isomers on two different bases of calculation is complicated, however, and can (and in this case does) become confusing. It did show that the infants, and inherently their mothers as well, are exposed differently according to isomer, parity and locality. It also indicates that there may be multiple routes of exposure, with mothers probably having different contact patterns with these routes, even in the same town. Therefore, at lower exposures (exposures other than IRS DDT), determinants other than maternal age, infant age and parity could result in stratifications of an otherwise presumed, homogenously exposed population, such as the mothers in Kwaliweni. Food such as fish and milk, or even water could be a source, but that would not likely account for the high o,p′-DDD levels found here, if DDT, applied only for malaria control outside Kwaliweni, was the only source. Plants such as cabbage and spinach, however, can convert p,p′-DDT to p,p′-DDD (WHO, 1979), and possibly also for the o,p′- isomers, raising the possibility of recent illegal use of DDT for local crop protection, although the low levels of p,p′ and o,p′-DDT in the samples mitigate this possibility somewhat. Longer residence times of o,p′ isomers (when compared with the p,p′ isomers) under Kwaliweni conditions might also contribute, as well as, somehow, preferential transfer of o,p′ isomers to Kwaliweni mothers via unknown routes and vectors. It would be very interesting and informative to derive a better understanding of the dynamics and kinetics of the o,p′ isomers with structured future studies.

### 4.5. The pyrethroids

Whereas the ultimate source of DDT would be from malaria control, pyrethroid residues in breast milk in this case probably have multiple sources. All the pyrethroids we analysed are used in agriculture, domestic applications and, in the case of deltamethrin, for malaria control. Contaminated drinking water and food might also be a route of exposure from any of these sources. Deltamethrin, used in this area for malaria control two seasons before, was only found in 31 samples, and all of them came from Jozini (Tables 2 and 3). Purely based on this profile, it is probable, but certainly not yet proven, that malaria control could have a contributory source of these residues in Jozini, as they were not found in breast milk from the other two areas. The low number of samples with detectable residues also indicates that deltamethrin (assuming deltamethrin applied as IRS as the major source)

is eliminated much faster than DDT. However, the malaria control is unlikely to be the only source of exposure – the town of Jozini is also located close to a major cotton production area, where pyrethroids are used on a significant scale. These small-scale irrigated and dry-land cotton farms provide job opportunities to many women and children, and uptake from here is therefore very likely (Bouwman, 1997). This is further corroborated by the presence of the other pyrethroids in the samples (Tables 2 and 3). Permethrin, cyfluthrin and cypermethrin were all found in some samples from Jozini, but with no discernable pattern. Only two Jozini primiparae and five Jozini multiparae samples had detectable levels of all four pyrethroids. Two primiparae and three multiparae had detectable levels of three different pyrethroids (any combination), while five primiparae and seven multiparae had two. This pattern of variable pyrethroid detection is most likely due to a similar variability in exposure to agricultural application, and probably household use as well. Since the level of knowledge regarding pesticides in general, and active ingredients in particular was limited, it was not possible to garner more knowledge from specific exposure to pesticides on lands. Observations by us, however, show that women and children do work on lands sprayed on the same or previous days, and constitutes a likely route of exposure, through contact, harvested food and inhalation (Bouwman, 1997).

The use of insecticides for household use is however limited mainly to the use of a dust formulation on home-grown vegetables, that contain, as active ingredients, permethrin and carbaryl. The presence of permethrin in breast milk from all three towns, where this formulation is available at local shops, is a good indication that this is a probable source. This immediately brings to the fore the possibility that the carbamate carbaryl could also be present in milk, and this should be investigated.

Although the overall number of samples that had detectable levels of any pyrethroid was much less than for DDT (91 vs. 152; Tables 1 and 2), and was obviously quite variable, the Anova showed that for ΣPYR there were no differences between any of the pairs (Table 3). With 73 samples containing detectable amounts of permethrin, this pyrethroid is probably derived from agriculture (either from working on lands or through home gardening). This aspect obviously needs further investigation, as carbaryl might also be involved. Overall, the indications are that if any of these pyrethroids are used in any sort of way (agriculture or malaria control) near people, residues will be present in breast milk, and hence represents an additional secondary source and primary route of exposure for infants. Although the half-lives of these pyrethroids in humans are not well known, it seems safe to assume that continuous presence of the ingredients in the immediate vicinity of people will result in a concomitant and continuous presence in breast milk.

### 4.6. Water as a possible vector in Jozini

It must be kept in mind that water source as a factor was not an initial part of this investigation, and therefore subjects were not selected accordingly, nor can it be assumed that water sources *per se* are comparable between the different towns. As explained before, the only town for which we had sufficient data was Jozini. Unexpectedly the difference between levels for those using piped water and those depending on the river showed significantly higher levels for certain DDT parameters in the river water group (Table 4), but not so for the pyrethroids. The most obvious factor would be that mothers depending on river water would probably also have had more-readily access to fish from the same rivers, since DDT is not soluble in water. Although it seems more logical, there are two mitigating considerations. (1) The DDT-isomer residue profile typically found in fish from this region (e.g. fish had a %p,p′-DDD of between 20 and 32% on mean ΣDDT levels ranging between 22.8 and 79.5 μg/kg wet weight for three different fish species (Bouwman et al., 1990a), compared with about 1.7% for the river-group milk, and 2.7% for the piped-water group milk from Jozini) is very much different than that found in breast milk. One would assume therefore that the breast-milk and fish profile would show some congruence, but the non-significant differences between the means of %DDT rather implied the same source and route of exposure for both these groups from Jozini. (2) Also, the levels measured in fish are not high enough to explain the levels found in humans (Bouwman et al., 1990a). There is therefore no simple explanation as to what caused this apparent disparity between the two water groups from Jozini, and it will certainly require more investigation, looking at social, economic and environmental factors of exposures and routes.

### 4.7. Infant risks associated with DDT

We will not attempt an exhaustive risk assessment on the levels of DDT we found in breast milk, mainly due to the dearth of information on the effects of DDT on human infants. An excellent review of the health risks associated with DDT is presented (WHO Europe, 2003). We will, however, attempt to calculate some parameters associated with exposure, and where these levels have been exceeded. The human Acceptable Daily Intake (ADI) level for ΣDDT is 20 μg/kg body weight (μg/kg/bw) established by the FAO/WHO in 1984 (Coulston, 1985; FAO and WHO, 2005). Based on this level, as well as more recent studies, the Provisional Tolerable Daily Intake (PTDI) for ΣDDT was set at 10 μg/kg/bw (FAO, 2005) in 2000 by the JMPR. More recently, the Maximum Residue Limit (MRL) set by Codex Alimentarius for extraneously-derived ΣDDT in milk was set at 20 μg/kg wm (FAO, 2005). In the present case, the data in Table 1, dealing with whole milk would be more appropriate. It is obvious that Jozini primiparae had the highest mean ΣDDT level at 238.23 μg/l wm. Just based on the food MRL, this is exceeded by a factor of almost 12. For the highest level, we found, the MRL was exceeded 77 times. In this group, only two mothers had levels below the MRL. The multiparae exceeded the MRl by a factor of 5.7. Twelve mothers from Mkuze, and 18 from Kwaliweni were below the MRL. Although this did not come as a surprise, we also need to take into account that we are dealing with infants exposed to these levels for up to two years. Since in

almost all cases breast milk constitutes the main food source to these infants, note should again be taken of this, when balancing the risk associated with malaria and exposure.

Since the ADI was set at 20 μg/kg/bw, we can make simple calculations, based on some assumptions, to determine whether these are also exceeded for infants. There are various ways of doing this and we chose the parameters that were used for this region before (Bouwman et al., 1990b), namely a mean infant body weight of 5 kg, and a daily intake of 800 ml of milk. For the maximum level the ADI was exceeded by 12.3 times; for the mean level for the Jozini primiparae the ADI was exceeded by 1.9 times. All the other groups were below the ADI. Since the PTDI was set at 10 μg/kg, and based on more recent data, the factors calculated above doubles (to 24.6 and 3.8 respectively), and the Jozini multiparae now also exceeds the PTDI by 1.8 times. The other two towns remain below this intake level, except for two mothers each from Mkuze and Kwaliweni who exceeded the PTDI slightly. What is also apparent, is the infants born to primiparae, are at a relatively-higher risk than the breastfed infants of the multiparae, especially in Jozini. Since the PTDI was derived from studies more recent than those for the 1984 ADI, and included numerous studies on toxicology (such as the effects of 3-methylsulphonyl-DDE), biochemistry and endocrine disruption, and with the application of a safety factor of 100 based on a derived NOEL of 250 μg/kg/bw for humans, this intake value carries more weight than the previous ADI, and should therefore be taken more seriously than before. Therefore, if a statement has to be made based solely on these levels, there would be enough well-motivated considerations to warrant a strong concern about the exposure experienced by the infants to these levels of ΣDDT. However, the threat of disease and death posed by malaria, in our opinion, far outweighs the possible or real negative effects of DDT intake at these levels.

### 4.8. Infant risks associated with pyrethroids

This study was the first to have demonstrated the presence of pyrethroids in breast milk from a malarious area, as well as (as far as we could establish) the highest measured pyrethroid breast-milk levels. Permethrin was the pyrethroid most often found in the samples, and was the only one present in milk from all three towns (Table 1). As mentioned before, permethrin is also a constituent of an often-used dusting powder used by many people in their own gardens, but might also have been used for in-house pest control. The MRL for permethrin is 100 μg/l wm, and the ADI is 50 μg/kg/bw (FAO and WHO, 2005; FAO, 2005). Not a single sample exceeded the MRL (Table 1), and for the sample with the highest permethrin level (85.12 μg/l wm), the daily intake was only 13.6 μg/kg/bw, also below the set ADI.

Cyfluthrin was present in 40 samples from Jozini, and only one sample each from the other towns. As far as we are aware, the probable source would be via exposure on lands, but this needs additional investigation. The MRL for cyfluthrin is 10 μg/l wm, and the ADI is 200 μg/kg/bw (FAO and WHO, 2005; FAO, 2005). Only one sample (from the same mother that had the maximum level of permethrin above) came close – with a level of 458.56 μg/l wm, this sample clearly exceeded the MRL, but the daily intake from this milk was less than the ADI, at 73.4 μg/kg/bw.

Cypermethrin was only found in Jozini samples, and once again, exposure to agricultural use is the most likely explanation as to why 36 women from this town had detectable levels. The MRL for cypermethrin is 50 μg/l wm, and the ADI is 50 μg/kg/bw (FAO and WHO, 2005; FAO, 2005). None of these parameters were exceeded in this group.

Deltamethrin was detected only in Jozini, with exposure via malaria control (more than a year before), and domestic and agricultural use as probable sources. The MRL for deltamethrin is 50 μg/l in milk, and the ADI was set at 10 μg/kg/bw (FAO and WHO, 2005; FAO, 2005). The MRL was exceeded in only one sample at 83.08 μg/l wm, and the ADI was exceeded slightly at 13.3 μg/kg/bw.

It seemed that the levels we found in breast milk were not from malaria control, but this needs confirmation. The sources and route(s) of intake for both DDT and the pyrethroids remain unclear, but are probably a combination of inhalation, domestic use, agricultural (on subsistence lands, cash crop plots and commercial farms), malaria control, and food.

### 4.9. Cumulative risk assessment

It is obvious from the above that many factors are at play, which, although previously recognised (Bouwman, 1997), have up to now not been adequately appreciated for IRS in malaria control. The levels of pyrethroids we found in this study, was not anticipated. The large contribution of pesticides now being used for crop protection in the same area where malaria control is practiced also needs to be accounted for. The advances that have taken place since the previous investigations in the 1980s in this area, have also added new insights (Ungváry et al., 2003), and in general adjusted the various levels of concern to lower levels of exposure, and therefore added to the weight of concern regarding the conditions we investigated. One way of advancing our understanding of the admittedly incomplete picture we have of the risks associated with the levels measured in this and other studies is to use cumulative risk assessment, based on the well-established health or environmental risk assessment for single toxic agents (International Life Sciences Institute, 1999; Daston et al., 2004). Cumulative risk assessment (CRA) characterises the risks associated with multiple agents via multiple routes, and taking on broad aspects such as uncertainty, variability, timing, combining toxicity data, interactions between agents, and exposure. The role of *a priory* assumptions are critical. The ILSI report states the following. ‘‘While interactions among combined synthetic pyrethroids could be inferred, these compounds are so readily detoxified that that it is improbable that at very low levels there would be exacerbation of effects by the presence of multiple synthetic pyrethroids.’’ (International Life Sciences Institute, 1999). Although the report continues with ‘‘However, as is true for the OPs, data regarding possible interactions of mixtures or pyrethroids at low levels

are lacking.'', we don't know what constitutes low levels in breast milk, and there seems to be a continuous presence of pyrethroids in breast milk, negating the assumed quick detoxification in the maternal body, and with even less of an idea of the same detoxification in the infant body. Granted that the ILSI report is probably mainly concerned with scenarios relevant to more developed countries, we should take real care when extending or assuming risk assessment findings, whether from risk assessments for single or multiple agents, to developing country scenarios. In our case we have found pyrethroids and DDT in appreciable levels in breast milk, and have inferred the possible presence of carbamates as well. The toxicant interactions between these classes of compounds in humans and infants are not well understood at all. However, to derive at a situation where a CRA can usefully be conducted and the risk managed, we feel that a number of data gaps, based partially on this study, and knowledge from elsewhere, should be addressed.

- We have only looked at four pyrethroids, but we know that more are in agricultural use. These levels could also be measured.
- The possible association of carbaryl with permethrin, which was the only common pyrethroid at all three towns, also needs investigation, as it now seems probably that this carbamate could also be present in breast milk. We have been unable to trace any published material on carbaryl, or other carbamates and even organophosphates in general use, in breast milk.
- The current insecticide used as IRS in KwaZulu-Natal is bendiocarb, also a carbamate (with DDT used in areas with resistant vectors). We were unable to trace any work on bendiocarb in breast milk, and it would therefore be incumbent to investigate its levels in this matrix.
- Since South Africa is now using pyrethroids only on a limited scale for malaria control, potentially exposed breastfeeding mothers should be assessed as to their breast milk levels.
- Longitudinal or multiple cross-sectional studies should be done to identify time trends, and to determine the seasonal effects of agricultural use. There are two growing seasons in some African countries, possibly increasing the exposure and uptake from this source.
- The source and route of DDT in a mountainous area such as Kwaliweni should be investigated. Since DDT has never been used there, long-range transport cannot be excluded.
- Water or fish as a possible source or vector should be investigated for those mothers relying on river water.

## 5. Conclusion

We need to realise that the situation described above is more or less experienced by millions of citizens in South Africa, and comparable numbers and conditions are also relevant in many other African countries. We have established the simultaneous presence of both DDT and pyrethroid residues in breast milk from a malaria-endemic area in KwaZulu-Natal, South Africa. The DDT levels were lower than previous studies, but the presence and levels of pyrethroids have added another level of complexity. Although we could not conclusively implicate pyrethroids via malaria control as a source of these pyrethroids in the milk, and agriculture in this case is probably the major source, future uses of pyrethroids as IRS will probably add to the body burden, and therefore also to breast-milk levels.

The unexpected high pyrethroid levels we found also have implications for other insecticides. We suspect that carbaryl might also be present in breast milk, implicating the use of carbamates in general as an alternative for malaria control. In fact, carbamates are already in use as IRS in Mozambique and elsewhere. Therefore, a multi-agent and multi-route approach will have to be considered when assessing risks, as the complexity of conditions determining exposure and uptake (among others) in developing countries increases. Situations in developing countries should not be merely considered as ''developing countries conditions'', but rather be recognised as a highly dynamic and adaptive system of coping to changing conditions through either choice or necessity. One of the aspects that might change later on, due to improved family planning and increased wealth could be the deferral of having first babies to a later age. This might add to infant risks, as more compounds will accumulate, and then be released via breastfeeding.

DDT as used in malaria control has a set of well-known risks, established through decades of research. This risk-set is more than acceptable, when compared with the deadly implications of malaria, especially when no other obvious alternative is available and practicable, given the environmental and disease vector conditions in many African countries. On the other hand, the risks associated with newer chemicals are less well characterised, as are the risks of exposure to both DDT and its replacement agents, at least for a transitional period. The risk implications for millions of people in this regard is so large, that the change to any alternative, given the extensive body of research already available, is such that a careful and well-considered set of investigations (including additional research to obtain pertinent data), be supported to allow risk assessments and CRAs. The further development of required data to support CRAs should be a priority in this instance. The Stockholm Convention, in an effort to reduce, with the eventual aim of elimination, the use of DDT, a number of caveats have been agreed upon (Anon, 2004). In the context of this investigation, the Convention indicates that the development or implementation of alternative malaria control measures, whether chemical or not, should consider human health and environmental implications. This investigation has shown that more work needs to be done in this regard.

Finally, it is very important to note that we have found no reason whatsoever to implicate breastfeeding as any less worthy of promoting as the best sole nutrient source for infants.

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