1. Introduction

The advance of chemicals in industry during the XX century gave rise to a number of highly aggressive compounds to human beings, and that altered the ecosystems balancing. Human population is inevitably exposed to environmental pollution through air-degraded products, water, the soil and food and their introduction into the food chain (Gomez et al, 2011).

The use of pesticides has been recognized and accepted as an essential ingredient in the modern agriculture for the control of pests, which damage crops and as a result, they produce a severe loss in food production. However, the extended use of pesticides, together with the inadequate behaviors of prevention and use of basic protection requisites will increase the probability of accidental intoxication in a notorious manner (Ntow et al, 2009), (Páez et al, 2011).

The estimated worldwide pesticides application is about 4 million tons (Elersek and Filipic, 2011) and according to Instituto Colombiano Agropecuario (ICA) Colombia produce 16.999.216 litters of herbicides, 6.392.387 litters of insecticides, and 19.690.293 kilograms of fungicides (ICA, 2010) during 2010.

Approximately 1.8 billion people worldwide are engaged in agriculture and it has been estimated that up to 25 million agriculture laborers have suffered non-intentional intoxications every year (Alavanja, 2008). In developing countries, pesticides are the cause of up to one million cases of intoxication and up to 20.000 deaths a year (Duran-Nah and Colli-Quintal, 2000).

Among the different pesticides used, 85% are used for agriculture applications and the remaining 15% are used in homes, gardens, business applications, public health and veterinary (Idrovo, 2000) (CEPIS/PAHO, 2005).
Certain works like agriculture or pests killing represent the biggest risks of acute intoxication, while there is a latent danger for the population at large in their food chain (Ospina et al, 2009) (Thundiyil et al, 2008).

The agricultural development model in Colombia is mainly based upon the use of agrochemicals and according data reported by the Public Health Surveillance System of Colombia (SIVIGILA), there were in 2008 6,650 intoxication cases for the use of pesticides followed by 7,405 cases in 2009 and 8,016 cases in 2010, being the organophosphoric and carbamate pesticides the principal reasons for intoxications (SIVIGILA, 2010). Such pesticides are widely used agricultural inputs, and they are esters of the phosphoric acid and the derivates thereof, and they share in common as a pharmacological characteristic, the action of inhibiting enzymes having esteracic activities, and more specifically, the inhibition of the acetylcholinesterase. They are easily hydrolyze and they have a low capacity of remaining in the environment (Palacios and Moreno, 2004), (Chakraborty, 2009), (Ntow et al, 2009).

Other pesticides under study are the organochlorated, which are persistent, lipophilic and very steady. They can be accumulated in ecosystems, causing many toxic effects on reproduction, development and immunological functions of animals (Waliszewski et al, 2005). They have been universally reported in the adipose tissue and human serum (Rivas et al, 2007), (Côte et al, 2006).

This study determined the biomarkers the inner dosages, exposure and effect caused by the use of organophosphoric (OF), carbamates (C) and organochlorated (OC) pesticides. The levels for these pesticides were established in a sample of tomato and the good agriculture practices were implemented for the crops of tomato, which afforded to assure the crop sustainable management and the perception of hazards on the pesticides adequate usage and management.

2. Materials and methods

A descriptive cross section study was done including 132 laborers of the tomato crop in the location of la Merced – Caldas, during 2009 and 2010. This study considered three phases: the first was the diagnosis to determine the biomarkers for the chosen pesticides. An analysis was made on pesticides residues in tomatoes as well as the characterization of the present productive systems of the crop through a participating rural diagnosis. The second phase was intervention to guide the demonstration plots implementation wherein the good agricultural practices (GAPs) were shown which were compared to other plots managed under a traditional production system. The process of intervention was assessed during the last phase.

A questionnaire was applied including variables social, demographic, occupational, clinical, toxicological and tomatoes consumption habits. The pilot study was carried out on 10% of the total of the sample, although they did not make part of the research.

Following the criteria of inclusion, all laborers engaged in tomato planting entered into the study, provided that they were permanent residents in the community, who had used OF, C and OC pesticides at least during the six months previous to the study, and also who volunteered to participate in the study.
For the analysis of biological samples two blood samples were taken, one with 5 ml heparin for the determination of acetyl cholinesterase (AChE) and pseudo cholinesterase (PChE) by the technique of Michel and Aldrige (Vorhaus and Kark, 1953) and another of 5 ml without anticoagulant for the analysis of OC pesticides in serum. For this group 12 different pesticides (α-BHC, β-BHC, HCB, heptachlor, oxychlordane, α-chlordane, i-chlordane, α-endosulfan, β-endosulfan, 4,4-DDE, endosulfan and 2,4-DDT) were considered, which were determined by gas chromatography with electron micro capture (EPA, 1995) reporting the levels found.

With respect to the sampling of tomato it occurred at the beginning, on the highest peak and at the end of the production stage of tomato. For each sampling unit a zigzag path by the crop was followed, harvesting a tomato every three places along the zig-zag, and then based on a quartering system, obtaining a sample of 1 kg per plot. For determination of pesticide residues OF and OC the internal method for extraction AR-NE-03 was used, based on the multiresidue S-19 extraction method of the German Convention (DFG, 1987), followed by the gas chromatographic analysis with flame photometric detector FPD and ECD electron capture. Meanwhile, for the determination of residues of N-methyl carbamate an internal method based on W. Blass and C. Philipowsky (Blass and Philipowsky, 1992) was used. The levels found for these pesticides were considered as contamination.

A descriptive analysis by frequency counting, central trends measures and dispersion was made for those continuous type variables, as some of the variables inherent to laborers, and environmental and biological measurements. Continuous variables were transformed to normalize them. We also explored possible relationships between some variables and they were crossed by constructing contingency tables. We used the Student’s t tests and chi square tests for the comparison of quantitative and categorical variables. Subsequently, we then performed a bivariate, stratified and logistic regression analysis. To compare results among laborers who worked in plots with GAP and traditional applications, the paired data test was used of Wilcoxon and Fisher for quantitative variables and the MacNemar test for qualitative variables. This study took into account Resolution 8430 of 1993 by the Ministry of Health, which classified this research as a minimal risk work. This study was approved by the Technical Committee of Research and by the Ethics Committee of the National Institute of Health.

3. Results analysis of the total population included in the study

3.1. Social and demographic variables

A total of 132 agricultural laborers were registered for the study, which were occupationally exposed to pesticides in the location of La Merced, belonging to the urban area 12,1% (16) and to the rural area 87,9% (116). Some general characteristics of the population are shown in Table 1.

With respect to gender, 90,9% (120) were men and 9,1% (12) women. We found a statistically significant difference between ages by sex p <0,05. As for affiliation to the social security system, 99,2% (131) of individuals in the sample had some form of social health security.
3.2. Occupational history

At the time of the interview, 100,0% (132) of individuals reported to be engaged in agriculture, of them, five laborers were enrolled in a GAP program, which abolished the pesticide use as compared with other five who followed the traditional practices.

The time of exposure to pesticides ranged from three months to 35 years (Table 1), we found a statistically significant difference in the time of exposure to pesticides among men and women (p = 0.006).

Regarding the frequency of spraying 78,1% (104) of laborers reported applying at least once a week and 21,9% (28) used to apply pesticides every 15 days or more. Other variables related to the pesticide use are shown in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants Standard deviation</th>
<th>Range</th>
<th>Intervals of confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) for both sexes</td>
<td>40,0 10,8</td>
<td>13 74</td>
<td>35,9-49,3</td>
</tr>
<tr>
<td>Men</td>
<td>39,7 10,8</td>
<td>18 69</td>
<td>37,7-41,6</td>
</tr>
<tr>
<td>Women</td>
<td>42,6 10,5</td>
<td>13 74</td>
<td>35,9-49,3</td>
</tr>
<tr>
<td>Time using pesticides (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>109,2 88,1</td>
<td>3 420</td>
<td>94,8 - 127,2</td>
</tr>
<tr>
<td>Women</td>
<td>70,8 73,1</td>
<td>4 240</td>
<td>55,0 – 147,9</td>
</tr>
<tr>
<td>Hours a day of application</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5 2,5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking while using pesticides, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (7,8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>118 (92,2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Population characteristics of location La Merced-Caldas, 2010.

In dealing about the storing of pesticides 95,5% (126) of laborers reported having an exclusive area and 19,5% (25) keep them indoors. On the use of personal protective equipment (PPE) a high percentage of laborers 96,2% (127) reported using some type of PPE, only 3,8% (5) did not use them, being the most frequent the use of the high heel boot (86,3%), and it is important to clarify that an employee may report using more than one element.

Laborers reported the greatest use of PPE related to protection of the body (120,5%), followed by protection of the lower limbs (93,9%), while the high boot the most common.

82,3% (105) of laborers said they were changing their work clothes at the end of the workday. The highest percentage of laborers 99,2% (131) washed their clothes at home and of these, 36 (27,3%) reported washing work clothes together with the rest of the family’s clothes.
<table>
<thead>
<tr>
<th>Types and trade names of pesticides used</th>
<th>Active ingredient</th>
<th>Toxicological category</th>
<th>Control group</th>
<th>Quantity of application (L)</th>
<th>Standard deviation</th>
<th>Number of applications per harvest</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organophosphoric Compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lorsban</td>
<td>Chlorpyrifos</td>
<td>Insecticide III</td>
<td>25,414</td>
<td>10,155</td>
<td>2</td>
<td>500,000</td>
<td>0.12</td>
</tr>
<tr>
<td>Tamaron</td>
<td>Methamidophos</td>
<td>Insecticide I</td>
<td>2,811</td>
<td>4,914</td>
<td>2</td>
<td>30</td>
<td>0.5</td>
</tr>
<tr>
<td>Monitor</td>
<td>Methamidophos</td>
<td>Insecticide I</td>
<td>8,334</td>
<td>45,64</td>
<td>1</td>
<td>250,000</td>
<td>0.12</td>
</tr>
<tr>
<td>Roxan</td>
<td>Dimethoate</td>
<td>Insecticide II</td>
<td>1,541</td>
<td>1,022</td>
<td>1</td>
<td>4</td>
<td>0.12</td>
</tr>
<tr>
<td>Sistemin</td>
<td>Dimethoate</td>
<td>Insecticide II</td>
<td>9,093</td>
<td>42,63</td>
<td>2</td>
<td>1,125</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Carbamate compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furadan</td>
<td>Carbofuran</td>
<td>Insecticide I</td>
<td>17,648</td>
<td>73,36</td>
<td>2</td>
<td>400,000</td>
<td>0.2</td>
</tr>
<tr>
<td>Roundup</td>
<td>Glyphosate</td>
<td>Herbicide IV</td>
<td>3,850</td>
<td>13,86</td>
<td>3</td>
<td>50,000</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Use and amount of pesticides studied and applied by agricultural laborers, in La Merced-Caldas, 2010.
An inquire was made on training on the safe handling of pesticides, being established that 74.2% (98) had never been trained, therefore, they had no knowledge about the use and handling of pesticides.

In relation to pesticide exposure, 74 (61.2%) reported having presented some symptoms at the time they were using them and of these 85.2% (63) did nothing with respect to this issue, or they self-medicated and only 14.8% (11) consulted a doctor. It was determined that Furadan was the pesticide causing most of intoxications to the population under study, being this toxicity category I (extremely toxic).

### 3.3. Use of pesticides

Pesticides reported by individuals in the sample evidenced that the most commonly used were the insecticides in OF group, of these Lorsban (chlorpyrifos) was the most widely used for a crop of tomatoes. The most frequently used toxicological category was II (highly toxic), followed by I (extremely toxic) (Table 2).

### 3.4. Clinical manifestations

The most frequently ailment reported was headache with 43.9%, followed by dizziness with 38.6%, weakness 36.4%, ocular burning 34.8% and redness of eyes with 31.8%. Grouped symptoms by systems most frequently found were in the central nervous system (95.5%) (Figure 1).

**Figure 1.** Distribution of systems, location of La Merced-Caldas 2010.
3.5. Biomarkers of internal dose, exposure and effect

Determinations were made for OC in 132 farm laborers, who showed an average level of 1.3 μg/L, with the highest values 4,4 DDE (mean = 3.4 μg/L, SD = 2.8, Minimum = 0.3, maximum = 13.5, IC = 2.9 to 4.0) and hepta chlorine (mean = 2.3 μg/L, SD = 1.0, = 6.1 Minimum, Maximum = 0.6, CI = 2.1 to 2.5). The average was obtained from the results of twelve OC pesticides, which perform the same mechanism of action.

Out of the total of workers, 45 (34.1%) showed inhibition of AChE enzyme in erythrocytes (mean = 0.84, SD = 0.020, IC = 0.80 to 0.88) and only one (0.8%) in plasma level (mean = 1.69, SD = 0.025, IC = 1.64 to 1.74). Of those with inhibition of the enzyme in erythrocytes, 14 (10.6%) individuals were below 25% with respect to the reference value.

4. Analysis of agricultural laborers included in the project demonstration plots

As specified in the methodology, 10 laborers were taken, 5 of which voluntarily agreed to participate in planting the tomato crop using the GAP, that is, pesticides were not used however a biological control was made. The remaining 5 laborers planted tomatoes as they were used to, using the pesticides frequently applied. The results shown below correspond to these 10 laborers who are involved in the project within the intervention phase.

It was seen that laborers of the parcels with GAP used more protection than those of conventional plots.

Plots were compared with GAP and traditional regarding the change of clothes at the completion of the workday and it was seen that laborers in both plots after receiving training in the proper use and handling of pesticides, successfully performed this activity, which became a protective factor.

4.1. Use of pesticides

Laborers who worked in traditional plots reported the use of seven pesticides, all of them being insecticides, of which six belong to the chemical group of OF and one belongs to C. The most frequently used pesticides were Lorsban and Latigo with 28.6% (4) each. Two of the seven employees, (28.6%) belonged to the toxicity category I, three (42.8%) to the toxicity classes II and two (28.6%) to the toxicity category III.

4.2. Clinical manifestations

With respect to manifestations grouped by systems, laborers who worked in the plots using GAP, showed more symptoms corresponding to organs of senses, while laborers in the traditional plot the referred symptomatology belonged to the central nervous system. In general, traditional plots laborers, had clinical manifestations more frequently in all systems, although there were no significant differences.
4.3. Biomarkers of internal dose, exposure and effect

With regards to biomarkers of exposure the presence of OC pesticide levels was found in serum, in laborers of both plots.

For OF and C the determination of the AChE enzyme showed some inhibition after the exposure in one of the five cases (20,0%) in conventional plots, while laborers belonging to GAP plots showed no inhibition. For plasma no inhibition of enzyme was seen in any worker.

4.4. Environmental samples

With regards to the samples of tomato, in crops were traditional practices were implemented, pesticide residues were found belonging to the chemical groups of OF (chlorpyrifos and phenthoate) and n-methyl carbamates (carbofuran and 3-hydroxycarbofuran), presenting the highest concentration of residues in OF. For plots with GAP the presence of residues of same pesticides n-methyl carbamates and chlorpyrifos was seen, being the only difference the finding of dimethoate. These active molecules were not in the formulation of products recommended by the agronomist for the control of pests and diseases.

Tomato production, had a statistically significant difference between plots with GAP and the traditional plots (p = 0,020) (Figure 2).

Figure 2. Comparison of production (kg of tomato) between production systems, in the location of La Merced-Caldas, 2010.
5. Discussion

Pesticides have been of great help to developing countries in their efforts to eradicate insects, endemic diseases and to produce adequate food (Alavanja, 2009), (Ecobichon, 2001). There is a controversy about the world’s dependence on these agents, due to their excessive use, volatility, long-range transport and eventual contamination of the environment (Ecobichon, 2001).

In Colombia, pesticide exposure has become a public health problem (Ministry of Social Protection, 2003) due to the higher demand in the use thereof and to the impact on the population health and the environment.

According to this study, all laborers who were hired, used to work in the agricultural sector and they were occupationally exposed to pesticides, most of which laborers came from the rural area, and 90.9% belonged were males, with a wide age range from 18 to 69, indicating that this is a working population and that young adults are the ones most commonly hired to carry out agricultural activities. Although laborers who qualified for the study, used to be informal workers, and many of them had no working contract, a high percentage (87.1%) of individuals in the sample belonged to the subsidized system of health, in other words, they had health coverage.

Concerning the time of exposure to pesticides, this exposure was considered as chronic, as workers had been exposed for an extended period of time, having an average of 9 years of exposure, which can result in long-term harmful effects, being men who have longer exposure times because they are mostly engaged to farming. This data is also supported by the spraying operations frequency, since about 70% of laborers applied the products at least once a week at an average of 5 hours a day.

In dealing with the storage of pesticides, a high percentage of laborers (95.0%) reported having an exclusive area, which reduces exposure to both the worker and his family.

For EPP, (personal protection elements) laborers used to perform agricultural activities wearing clothes for work exclusively, being the high-leg boot the most commonly used item, as well as the disposable mouth masks. It is Important to point out that EPPs used by laborers were not commensurate with the risk they were exposed to, as for example, wearing face masks is not a proper practice for their protection as they have to handle these harmful chemicals, as they allow the entry via inhalation of pesticides, and it was also found that only a minority of laborers use gloves, allowing the entry of these substances by the dermal route, especially of those products having the characteristic of being lipo soluble pesticides such as OF and C. The parts of the body that were mostly protected were the trunk and lower limbs, being the upper limbs the ocular and respiratory regions the least frequently protected.

Although laborers reported that they changed their work clothes at the end of the workday, they washed their clothes at home mixing it with the clothes of the rest of their family, exposing their family members to intoxication risk by such substances.
We inquired about the training courses that laborers have received at some point in their working lives on topics like the safe handling of pesticides and it was found that 74.2% had never been trained, so they did not have the necessary skills to handle such substances.

Only a small percentage reported to see a doctor when they showed some kind of symptoms when they used pesticides, while others took home remedies or they medicated themselves which results in an underreporting of cases of poisoning by such substances, as the intoxication cases are not reported to the system of public health surveillance in Colombia (SIVIGILA).

A high number of pesticides is used in Colombia primarily in categories I and II toxicological categories, and by chemical group the OF and C. This information is confirmed by other studies conducted in Colombia (Varona et al, 2007, 2009), (SIVIGILA, 2010), thus increasing the chances of triggering effects on health. Among the clinical manifestations reported by laborers, most of them are related to neurological and sensory organs disorders. Neurological disorders, may be related to pesticides OF and C, while manifestations of sense organs can be triggered by the use of multiple chemicals, including pesticides that are the subject of this study. The same occurs with the manifestations of the digestive system, which can onset by the ingestion of different chemicals, although they can also have a bacterial and viral origin, among other causes.

This study used biomarkers of internal dose, exposure and effect, which allowed setting the pesticides levels in 132 biological samples. OC pesticides which were most frequently found in biological samples were 4,4-DDE and endosulfan. It is important to point out that laborers did not report the use of these pesticides in the tomato crop, so the presence of these is explained by the environmental pollution and toxicokinetics inherent to this group of pesticides. Although OC were banned in the country since 1993 due to their high persistence, their ability of bio-magnification and their neurotoxic effects, they were still used for about 40 years.

The determination of enzyme AChE continues to be widely used to measure the exposure to OF and C, however, interpretations of results are highly variable, since there are genetic and physiological causes as well as associated pathologies, which can decrease the levels of this enzyme (Varona et al, 2007).

In addition, there is a significant variation within the same individual, therefore, the medical surveillance of laborers continuously exposed to these two groups of pesticides must also include not only the medical examination, but also the determination of enzyme AChE pre-exposure (baseline) and quarterly for the duration of the exposure (Varona et al, 2007).

This study reported that 34.1% of all laborers showed inhibition of this enzyme in erythrocytes, which confirms the fact that OF and C compounds are the most used by the laborers included in the study.

Within the research project and as stated earlier in the methodology, a second phase known as intervention was conducted. During this stage 10 individuals were included, who had some schooling, which allowed them to gain a better understanding of the concepts used in training on GAP and thus the implementation thereof was facilitated.
It was found that laborers used pesticides in plots with GAP, but his recommendation was not given by the agronomist, while in conventional plots they reported the use of pesticides inhibiting the AChE, which are classified as extremely and highly toxic causing a large exposure to this chemical group of pesticides, which in turn affect the central nervous system, a situation that is related to the clinical manifestations reported by laborers in the study of these plots (Idrovo, 2000), (Cassarett and Doull, 2005) (Goldfrank et al, 2006). Regarding the use of EPP, laborers of parcels with GAP used more protection, especially respiratory and eye protection than the conventional plots.

The analysis of biomarkers of exposure and effect for laborers who participated in the intervention phase showed the presence of OC pesticide levels for both traditional plots for GAP. None of the laborers reported the use of these pesticides in the tomato crop, as stated in the diagnostic phase, and their presence is due to their high persistence and their ability of bio magnification. Despite of the fact that their use is prohibited in Colombia, it is not uncommon to identify patients with acute and chronic effects resulting from the exposure to this type of pesticides (Varona et al, 2010), (Córdoba, 2006).

The determination of enzyme AChE showed the after exposure inhibition in a worker of conventional plots, however, this reduction did not require any treatment but a medical surveillance. It is noteworthy to say that there was not a greater number of laborers with inhibition of AChE enzyme considering that in conventional plots the use of OF and C pesticides was reported. This can be explained because this group of pesticides are easily hydrolyzed and excreted by renal way, and there is no bioaccumulation or bio magnification, but this is also due to the fact, that the specimen taken for the determination of the enzyme AChE, should take place within 24 hours after the exposure as a maximum. Since this enzyme subsides to the exposure, it starts to regenerate and therefore, it cannot show the true percentage of inhibition.

When comparing the production of tomato obtained by the two crop systems, it is seen that the range of average production of crops following the traditional practices were very similar to the crops following the GAP. However, when comparing the average tomato production, crops with GAP showed a statistically significant difference higher than the results attained by the traditional system. This behavior can be explained because the fertilization plan in plots using GAP satisfactorily met the needs of the soil.

Regarding the pesticides residues that followed traditional practices, we have found that OF showed the highest levels of residues in the initial stage of crops, where chlorpyrifos was the highly concentrated pesticide but it did not exceed the Codex maximum residue limits (MRLs). For crops established with GAP, we found that five crops presented residues of pesticides the active molecules of which were not recommended by the agronomist for the control of pests and diseases. It was also found that the highest concentration of residuals detected were due to the use of OF.

Although there was no evidence of commitment to health in terms of the effects assessed in this study, we detected significant correlation with respect to one of the traditional plots. It
was reported for that plot that one of the individuals presented cholinesterase inhibition and coincidentally it is the plot where the residues in tomato reported the presence of chlorpyrifos. This is a situation of high concern, because this means that farmers in this area are significantly applying pesticides at this time.

The above implies that there is no risk perception by farmers, which makes it necessary to carry out educational campaigns to warn them about the need of at least meeting the rules and procedures laid down for each product. It is recommended to continue with the follow-up and support to this type of population through training and sensitization of laborers in an attempt to reduce the pesticide use and as a result to reduce the effects on their health from exposure to such substances.

It is necessary to strengthen farmers in the implementation of GAP and the advantages of this production system.

The use of chemical pesticides should be performed following the manufacturer's technical recommendations such as presence of pests, application dose, frequency of application and exhaust period in the context of an integrated pest management program which reduces the risk of finding concentrations exceeding the MRL which implies some weakness of competitiveness of crops.

6. Funding sources

This research project was funded by Colciencias, the National Institute of Health, the Colombian Agricultural Institute, University of Valle and Caldas Territorial Health Department.

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Acknowledgement

We want to express our gratitude to the workers participating in this study. To Dr. Natalia Carvajal of University of Quindio for their contributions to this research. To Drs. Nelcy
Rodriguez and Viviana Rodriguez, for their statistical analysis of information. To Hernán Correa and Jhon Jairo Gonzalez of Caldas Health Territorial Department, for their assistance to create the brochure as well as in field assistance and Jose Gabriel Muñoz, for his part in finding workers for the study.

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