Assessment of Children's Pesticide Exposure Through a Visual Time Activity Diary

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Executive Summary

Mancozeb and maneb belong to a class of fungicides that are widely used in agriculture world-wide. In Costa Rican banana plantations, these fungicides are sprayed on banana plantations via light aircraft. Animal toxicity studies suggest that maneb and mancozeb can be carcinogenic but there are few human studies on exposure or health effects. The main metabolite of these fungicides is ethylenethiourea (ETU), which has also been shown to be carcinogenic in animals and has the potential to adversely affect thyroid function in humans. Physiological and behavioral reasons make children more vulnerable to pesticide exposures. The aim of this study was to examine the relationship between child time activity patterns and levels of urinary ETU in children that live on banana plantations sprayed with mancozeb and maneb in Costa Rica.

Children in the study (n = 37) were recruited from a small banana plantation community in the Caribbean coast of Costa Rica. Urine samples (first morning void) were collected once daily for 7 days and children were also asked to complete a visual time activity diary for each of the 7 days. The time activity diary was a 4 inch x 6 inch card with pictures that the children wore around their necks during the day. It was pilot tested for one week and changes were made according to the children's reactions in preparation for the final study. The urine samples were frozen and mailed to Lund University in Sweden where they were analyzed for ETU levels in µg ETU/g creatinine, or µg/L ETU adjusted for density.

Compared to levels of ETU reported in other studies, we found that children living in the banana plantation are highly exposed to ETU but this varies according to their activity patterns and whether aerial spraying occurred or not. For example, active children in this population had on average have 1.7 times higher levels of ETU in their morning urine after spraying days as compared to days where spraying did not occur. There is evidence to suggest that children have higher levels of ETU in their urine after spraying days if they spent 7 or more hours being active and outside.

Implementation of the time activity diary was possible with this particular group of children but the accuracy of the responses should be validated with external observations of the children. The validation through external observations verifies the responses recorded by the children and is an important step when working with new groups of children.

Abstract

Background:
Mancozeb and maneb are in a class of fungicides that are widely used in agriculture world-wide. In Costa Rica, these fungicides are sprayed on banana plantations via light aircraft. Animal toxicity studies suggest that maneb and mancozeb can be toxic but there are few human studies in regards to the health effects from exposure to these fungicides. The main metabolite of these fungicides is ethylenethiourea (ETU), which has also been shown to be carcinogenic in animals but not completely determined in humans.

Objectives:
We investigated the relationship between child time activity patterns and levels of ETU in children that live on banana plantations sprayed with mancozeb and maneb in Costa Rica.

Methods:
Children in the study (n =37) were recruited from a small banana plantation village in the Caribbean coast of Costa Rica. ETU levels were measured in child urine. Urine samples (first morning void) were collected once daily for 7 days and children were also asked to complete a visual time activity diary for each of the 7 days.

Results:
All children had detectable ETU levels in the urine samples. The geometric mean for ETU for all children from the study is 5.5 µg/L ETU with a range from 1.2 – 19.7 µg/L, density adjusted. The geometric mean (GM) for ETU in the urine samples was 6.96 µg ETU/g creatinine (range= 0.96 – 25.98 µg ETU/g). The 95% confidence interval (CI) for the GM was 1.75 µg/g – 1.93 µg/g. The only categories with sufficient data were [active, outside] and [quiet, inside], since children were rarely reported being [quiet and outside] or [active and inside]. Children who were active and outside for 7 or more hours had on average 1.7 times higher levels of ETU in their first void urine the day after spraying occurred as compared to days when no spraying occurred ($p<0.01$). When comparing children who were active and outside 7 or more hours on days when spraying did and did not occur, there is evidence to suggest that urine ETU levels are higher on days where spraying occurred ($p = 0.007$).

Conclusions:
We report that children living in the banana plantation are highly exposed to ETU and this varies according to their activity patterns and whether aerial spraying occurred or not. Implementation of the time activity diary was a success with this group of children but the accuracy of the responses should be determined with external observations of the children.

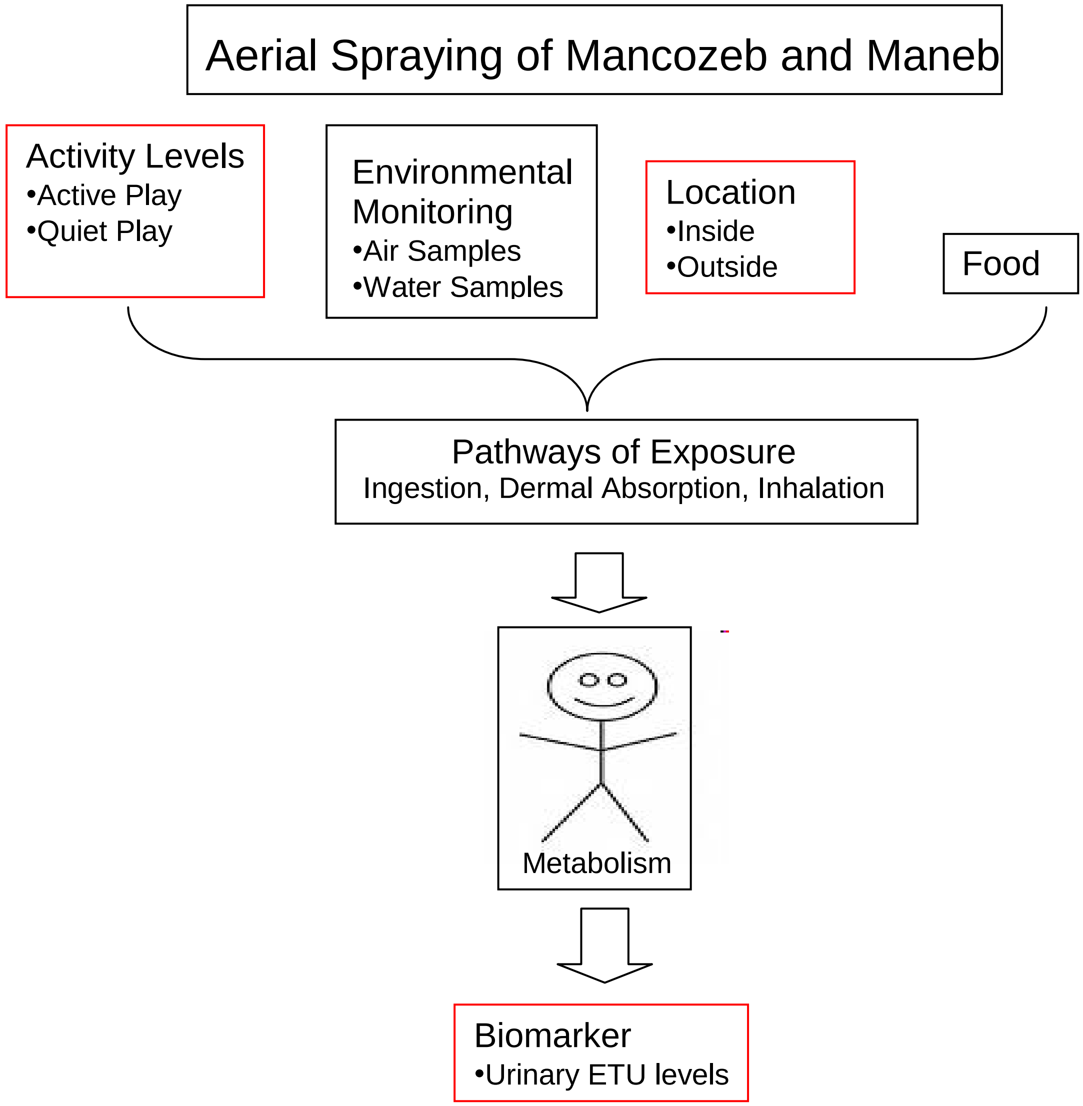
Introduction

Children are widely exposed to many different kinds of pesticide contamination in residential and school environments where they spend their time.
As chemical substances can negatively impact their health and development, it is important to develop methods to assess the levels of exposure and to determine the factors that influence these exposures (Hubal et al. 2000). In a review article of several children's studies, Fenske et al. (2002) indicates that the best way to assess children's pesticide exposure is by using a combination of biomarkers, environmental monitoring, and activity monitoring.

Children differ from adults physiologically and behaviorally in that they have a high surface area to body weight ratio and engage in frequent hand-to-mouth contact, all of which increases their exposure to harmful substances in their environment. Children engage in activities and spend time in different microenvironments which also influence their exposures. Exposure is the contact that an individual may have with a pollutant of interest over a certain period of time. These exposures lead to toxicants passing into the body through the pathways of ingestion from food/water, inhalation, and dermal absorption. With a larger body weight to surface area ratio than adults, dermal contact becomes a more important pathway for exposure in children than in adults.

Figure 1 below shows the pathway of children's exposure to aerial spraying of pesticides and the resulting biomarker of interest. Urine sampling is used to measure chemical exposure by analyzing for the metabolite of interest. It is important to remember that urinary metabolite levels are only an indicator of exposure and is not a direct measure of dose. To figure out the dose (defined as milligrams per unit of body weight) that a child was exposed to, one would also need to know the rate of elimination of the metabolite from the body to the urine and the fraction that is excreted in urine.

Figure 1. Pathway of Exposure for children. Boxes in red were focus of study.



Monitoring of a child's time-activity patterns can yield information as to where children spend most of their time which can lead to more information about pathways of exposure. However, activity monitoring is often the most difficult part of the exposure assessment because it can be time consuming, costly, and inaccurate (Hubal et al. 2000). Some time activity monitoring methods include videotaping children, placing geographic positioning system (GPS) monitors on children, and creating time activity diaries for parents to complete for the children (Elgethun et al. 2007, Ferguson et al. 2006). A *visual* activity diary divides a 24-hr period into one-hour increments and is composed entirely of pictures to promote simplicity and clarity to children completing the diary.

Children from agricultural communities are at a particularly high risk for pesticide exposure because they might live in close proximity to the fields that are being sprayed with pesticides and they can also be exposed from parents that bring home pesticide residues on their clothing. Many studies have looked at children's exposures to organophosphates. Eskenazi et al. 2007, for example, reported that prenatal dialkyl phosphates (DAPs) were associated with neurobehavioral developmental problems in children. Another study in the same cohort found that early exposures to organophosphates, endotoxins, and allergens in an agricultural community were associated with increases in biomarkers of allergic asthma (Duramad et al., 2006).

Another class of pesticides that is less studied is fungicides including compounds called ethylenebisdithiocarbamates (EBDC). The EBDC group includes zineb, maneb and mancozeb all of which have low acute toxicity and degrade quickly (Jacobsen et al. 1997). According to Fustoni et al. (2008), over 21,000 tons of EBDCs were used in Europe in 2006 because of their low acute toxicity and effectiveness in removing fungal diseases. Routes of exposure for EBDCs include dermal, ingestion and inhalation. Mancozeb is a fungicide commonly used in aerial spraying of fruit trees like apples, pears and bananas as well as on vineyards and potato fields (Fustoni et al. 2008).

The main metabolite from all EBDCs in the human body and from environmental degradation is ethylenethiourea (ETU) (Colosio 2002). ETU also exists as an impurity in EBDC formulations of mancozeb and maneb. ETU is a highly polar and stable compound, and is soluble in water (Jacobsen et al. 1997). ETU does not bioaccumulate in organs, but its half-life does vary among different species. In rats and mice, ETU can be cleared from the animal in less than 10 hours, but in humans it takes about 24-48 hours. In animal toxicity studies, ETU is known to interfere with thyroid hormone activity and found to be teratogenic and carcinogenic in rodents via a non-genotoxic route (Cecconi 2007). Steenland et al. (1997) found that there was an increase in thyroid stimulating hormone in workers whose main job duties came from pesticide application compared to the control group ($p = 0.05$). A study by Panganiban et al. (2004) conducted a biomonitoring study on banana plantation workers in the Philippines by taking blood ETU samples. This is the first study that looked at blood ETU levels and urine ETU levels. They found a strong correlation between the sizes of solitary thyroid nodules and blood ETU level ($p = 0.001$). In 2003, the International Agency for Research on Cancer (IARC) downgraded ETU's classification from a group 2B to a Group 3 chemical, citing that there is not enough evidence from human studies to classify ETU as a carcinogen but sufficient evidence in (IARC Monograph 2003).

Studies on ETU have mainly been conducted by Italian researchers on occupational and general populations both in rural and urban areas because of the widespread use of EBDC's in Italy (Aprea et al. 1997). Due to the small number of studies on ETU and lack of a good reference value it has been relatively difficult to completely understand the levels of ETU collected in a biomonitoring study (Colosio et al. 2006). Reference values are useful because it provides a level of exposure of the general population to which other biomonitoring results can be compared. ETU has been detected in urine samples of both the general and occupational populations. Aprea et al. (1996) examined urinary ETU levels of the general population in four different regions of Italy and found that ETU ranged from 0.8 - 8.3 µg ETU/g creatinine in urban areas compared to 0.9 – 61.4 µg ETU/g creatinine in rural areas where spraying occurred. In a more recent study, Colosio et al. 2006 determined that reference values for ETU in Northern Italy ranged from 0.6 – 0.8 µg ETU/g creatinine for $n = 95$ healthy individuals. A study by Aprea et al. (1997) investigated the exposure for five healthy, non smoking male students and their exposure to ETU from a controlled diet. The study found that ETU levels increased with consumption of fruit, vegetables and wine, with ETU levels ranging from 0.6 - 6.7 µg ETU/g creatinine.

Biological monitoring for ETU exposure in occupational settings has included vineyard workers, pesticide applicators, potato farmers, banana plantation workers, and even pesticide formulation workers. Table 1 presents current literature that measured urinary ETU levels in different populations, the ranges of ETU measured, as well as the methods used. Among these studies, the highest levels of urinary ETU were found in workers of a pesticide factory that manufactures EBDCs (Aprea et al. 1996). The highest levels of ETU came from those involved in pesticide formulation (0.3 - 644.4 µg/g).

Relatively few studies measuring ETU have been conducted outside of Italy but there are many communities world wide where EDBCs are heavily used. One such area is the banana plantation in the Talamanca region of Costa Rica located on the Caribbean coast. Our study aims to look at mancozeb exposure as assessed by ETU levels in relation to time activity patterns of school-age children who lived on banana plantations in this area. The children from that community are potentially highly exposed to manozeb and maneb from the aerial spraying of the banana fields. Their homes and schools are located in close proximity to the banana fields (in some instances less than 10 meters) and the children often play in banana fields unsupervised or are brought into the fields with their parents on weekends.

Table 1. Review of ETU urine biomonitoring studies in general and occupational populations.

Author (year)	Population	Method	% Detection	ETU level* (mean/range)
Aprea et al (1996)	**Italy** General	Collected samples during medical checkups		
	n=97 rural area		37	GM: 2.1 (0.8 - 8.3 µg/g)
	n=167 urban area		24	GM: 5.3 (0.9 - 61.4 µg/g)
Aprea et al (1997)	**Italy** General	Collected 24 hr and 8 hr samples after special diet consumption for 8 days		
	n=5 non smoking males			Mean: 3 (0.6 - 6.7 µg/g)
Colosio et al (2006)	**Italy** General	Collected spot sample in the morning		
	n=95		60	0.5 - 11.6 µg/g
Saieva et al (2004)	**Italy** General	Collected 24 hr sample after food consumption		
	n=69		21.7	GM: 5.5 nmol ETU/day
Aprea et al (1998)	**Italy** Occupational n=57 workers in pesticide formulation plant	Collected sample once on last day of week for 2 weeks		0.3 - 644.4 µg/g
Colosio et al (2002)	**Italy** Occupational	Collected 50 ml spot urine sample before and after work shift		
	n=13 vineyard workers		100	0.5 - 9.2 µg/g
	n=13 control		0	< 0.5 µg/g
Steenland et al (1997)	**Mexico** Occupational Back pack pesticide sprayers	Collected 1st morning sample day after exposure		
	n=49 heavy exposure			GM: 19ppb
	n=14 light exposure			GM: 9ppb
	n=31 control		0	NA
Fustoni et al (2008)	Occupational	Collected 1 sample at T=0 days and 1 sample at T=30days		

	Bulgaria (2 studies)			T=0 days (mean and range)	T=30 days (mean and range)
	n= 55 green house workers			5.9 (<0.5 - 73.6 µg/g)	182 (<0.5 - 983.2 µg/g)
	n= 45 controls			24.9 (<0.5 - 327.2 µg/g)	114.4 (<0.5 - 166.7 µg/g)
	n= 52 employees in zineb factory			34.8 (<0.5 - 173 µg/g)	58.4 (<1.7 - 868.3µg/g)
	n= 49 controls			1.4 (<0.5 - 16.5 µg/g)	NA
	Italy				
	n= 48 vineyard workers			1.8 (<0.5 - 37.1 µg/g)	14.9 (<0.5 - 62.5 µg/g)
	n=45 controls			1.3 (<0.5 - 8.1 µg/g)	NA
	Finland				
	n= 51 potato farmers			<0.5 (<0.5 - 2.4 µg/g)	45.4 (<0.5 - 428.6 µg/g)
	n= 49 controls			0.9 (<0.5 - 11.6 µg/g)	<0.5 (<0.5 - 4.6 µg/g)
	Netherlands				
	n=42 floriculture workers			1.3 (<0.5 - 7.3 µg/g)	1.1 (<0.5 - 2.7 µg/g)
	n= 40 controls			1.2 (<0.5 - 4.8 µg/g)	1.2 (<0.5 - 5.5 µg/g)
Colosio et al (2007)	**Italy** Occupational	Collected 1 sample (2nd morning void) in the morning at T=0 day and T=30 days		T=0 days (mean and range)	T=30 days (mean and range)
	Pavia				
	n=14 vineyard workers			4.2 (<0.6 - 37.1 µg/g)	23.5 (3.1 - 62.5 µg/g)
	n=11 control			1.4 (<0.6 - 8.1 µg/g)	NA
	Trento				
	n=34 vineyard workers			<0.6 (<0.6 - 3.1 µg/g)	11.3 (<0.6 - 42.8 µg/g)
	n=34 controls			1.2 (<0.6 - 5.1 µg/g)	NA
Phung et al (2008)	**Costa Rica**	Collected 1st morning void sample once every day for 7 days			
	n=37; 7-10 yr old children living on banana plantations		100	GM: 5.72 (1.2 - 19.4 µg/l) GM: 6.96 (0.94 - 25.98µg/g)	

* Levels in µg/g are µg ETU/g creatinine.

Methods

Study Population

The study was conducted in a banana plantation community in the Limon Province of Costa Rica. This particular area was chosen because it had participated in a previous study by the Universidad Nacional – Heredia, Costa Rica in 2007 and it was found that the children in that rural area had the highest levels of ETU among all children sampled in several communities (van Wendel de Joode et al. 2008). The main eligibility criteria for selection of the study population were that the children from the community had participated in the study from 2007. Of the 51 participants that were in the 2007 study, we enrolled 17 girls and 20 boys aged 7 – 10 years old, for a total of 37 participants in the 2008 study. The children were in the 2nd – 4th grades except for three children who were still in 1st grade. Approval of all procedures was obtained from the University of California, Berkeley Institutional Review Board (IRB) and from the Ethical Committee of the Universidad Nacional - Heredia before the field work began. Informed consent as well as children's assent was received from each participant and their parents. All sampling for the study occurred between mid – June and mid – August 2008. This study was completed in partnership with Lund University of Sweden and Universidad Nacional – Heredia, Costa Rica.

Data Collection and Study design

Visual Time Activity Diary

The original diary was designed by Dr. Brenda Eskenazi's research group at the Center for Children's Environmental Health, University of California, Berkeley (Bradman et al. 2007). It was originally designed for mothers in the farming community of Salinas, CA to complete for their young children aged 12 – 24 months. The diary was adapted for this study so that older children (7 – 10 years old) can complete it for themselves. Some of the aspects that were taken into consideration for the design of the visual time activity diary included age of participants, culture, and weather. Additionally, the diary for this study had to be designed for convenience as well as durability since children in this Costa Rican community often play outside and are subject to the heavy downpours of the Caribbean coast. Given the different cultural contexts between Salinas and Costa Rica, we modified the pictures so that a child could associate a picture with the same meaning as children in the Salinas Valley. After an initial assessment of their physical environments and activities, a pilot diary was designed. The first page of the diary was the list of activity options – sleeping, watching television, eating, quiet activity, active activity or other activity not listed and their corresponding numbers. The remaining three pages were divided into morning, afternoon, and evening hours. Each of the three pages had a column for the hour of day, a blank column to write in the number of the corresponding activity as well as four options to circle for location – home, classroom, outside, or banana plantation (Appendix A). These microenvironments were chosen as defined by Hubal et al. (2000).

After pilot testing the initial version of the diary, many changes were made and a simpler version was designed to be the final version of the diary (Appendix B). The activity options were simplified to a choice between quiet activities or active activities because it was less confusing for the children since most activities could be placed in to either category. Quiet activities included sleeping, eating, watching television; reading etc. and active activities included playing sports, running, cleaning etc. Instead of having to write down the corresponding activity number, the child could simply circle the corresponding number on the diary. The location options were also limited to inside, outside, and banana plantation. Instructions for the diary completion were to complete it individually and hourly, as well as circle the activity that the child did for the majority of the hour.

The physical form of the diary remained the same throughout and consisted of four laminated pages 4 inches by 5 inches and bound together at the top left corner with a key chain ring. The 1st page showed the list of activities, and the 2nd, 3rd, and 4th pages corresponded to the morning, afternoon, and evening pages that were to be completed respectively. Attached to the key chain ring were a long cord and a pen so that the children could wear the survey around their necks with the pen attached.

Pilot Testing

During the pilot study, all participating children were first trained in small groups on how to complete the diary and again individually for reinforcement. The first version of the diary was piloted to test for ease of understanding instructions, accurate completion and to assure that its completion did not interfere with their normal daily activities. We also visited the homes of the participants to explain to their families the study objectives and asked for their help in reminding the children to complete the diary accurately. No urine samples were collected during the pilot study.

Final Study

Table 2 presents the data collection scheme. On Day Zero, each participant was given a time activity diary and instructed to complete it. From Day Zero onward, blank diaries were always passed out the day before it was to be completed. On Day 1 of the study, each participant was given the diary for Day 2 and also given a zip lock bag with a small plastic bottle of approximately 20 ml to bring home for their urine sample. Urine collection began on the Day 2 of the study when children were asked to bring the first morning void in the plastic bottle to school and Day 2 urine samples reflected exposure on Day 1. On Day 2 of the study, the diaries from Day 1 were collected and blank diaries for Day 3 were distributed. This sequence of collecting diaries and urine samples continued for 7 days. Even though urine samples reflected exposure from the day before, this collection scheme allowed us to keep the results of the Day 1 diaries and the Day 2 urine samples together as a bundle, so during analysis, it was not necessary to think about which urine samples referred to the day before. If a child forgot to bring their diary or urine sample, investigators would collect it from the child's home. We also made random observations of the children's location to compare with their diary responses for validity of the diary as a tool for monitoring how children spend their time.

Table 2: Sample collection scheme.

Day of Study	Item Distributed		Item Collected	
Day 0	Day 1 diary	None	None	None
Day 1	Day 2 diary	urine bottles	None	None
Day 2	Day 3 diary	urine bottles	Day 1 diary	Urine samples*
Day 3	Day 4 diary	urine bottles	Day 2 diary	Urine samples
Day 4	Day 5 diary	urine bottles	Day 3 diary	Urine samples
Day 5	Day 6 diary	urine bottles	Day 4 diary	Urine samples
Day 6	Day 7 diary	urine bottles	Day 5 diary	Urine samples
Day 7	None	urine bottles	Day 6 diary	Urine samples
Day 8	None	urine bottles	Day 7 diary	Urine samples

***Urine samples reflect exposure from the day before.

Validation of Children's Responses

To validate the children's diary responses with regards to their location, our team of Swedish and Costa Rican researchers completed 216 random observations over the seven day study period of the children's locations. Observations of the children's activities were not recorded because it was not possible to observe a child's activity when he or she was inside without disrupting their normal behaviors. Children were observed anywhere from one to twelve times over the course of the study period. We compared our observations of the child's location with the child responses on the activity diary. The external observations were conducted by walking through the village neighborhoods and making notes of children's locations with as little contact with the children as possible. If children were outside, that was easily noted and if they were not, we would ask the family if a particular child was inside or not at home. We also asked a school employee to help make external observations in her vicinity because she lived near some of the children.

Urine Collection and Analysis

One morning void sample was collected from each child for 7 days. If a child forgot to bring in the morning urine sample, a spot sample was collected. In addition, some children were asked randomly to give spot samples with his or her consent in addition to the morning urine sample. Spot samples were only taken at school so we knew the exact time of collection. After receiving the urine samples from the child, it was poured into a smaller test tube for storage. ETU is known to degrade quickly in light (Aprea et al. 1996), so all tubes were immediately placed into an ice chest for storage until we could place it in the freezer at our research site. Frozen urine samples of 15 ml aliquots were sent with dry ice via FedEx to Lund University in Sweden for analysis by Dr. Christian Lindh's laboratory. The analytical procedure for extraction of ETU from the urine samples used liquid chromatography triple quadruple mass spectrometry (LC/MS/MS). The limit of detection (LOD) for ETU was set at 0.05 ng/ml. Further details of this method can be found at Lindh et al. (2008). ETU levels were adjusted for both creatinine levels and density to account for varying levels of creatinine and urine volume excreted by each individual.

Statistical Analysis

Based on the responses collected, we determined for each child the number of hours the child spent in each of the following four microenvironments: active and outside, active and inside, quiet and outside, and quiet and inside for each day of the study. The only categories with sufficient responses were [active and outside] and [quiet and inside], since children were rarely reported being quiet and outside or active and inside. Spot urine samples were not used for this analysis. Urinary concentration of ETU (creatinine adjusted) was log transformed to yield a normal distribution and all statistical analyses used the log transformed value. Multivariate linear regression models were created by making active & outside an indicator variable. Active and outside ≥ 7 hours was '1' and active and outside < 7 hours was '0'. A cross product was also created to test for interaction between days of spraying and being active and outside ≥ 7 hours. Three regression models were created with different baseline parameters to examine if different activity levels and occurrence of pesticide spraying contributed to higher levels of ETU. The models allowed for the comparison of mean difference in ETU levels for active and less active children on days where no spraying occurred, active and less active children on days where spraying occurred, and active children only on a day of spraying versus a day of no spraying. A fourth regression model was created to show how the relationship between age and gender may affect the outcome of ETU levels. After an activity diary was completed for Day 1, it was not collected until the following day along with the urine sample that reflected exposure from Day 1. Therefore, both components reflect activities and exposure on the same day. The models were all clustered by child to account for any intra-variability within the same child. The clustering helps control variable issues within the same child that might affect the overall standard error. All statistical analysis was completed with STATA 10.2 statistical software.

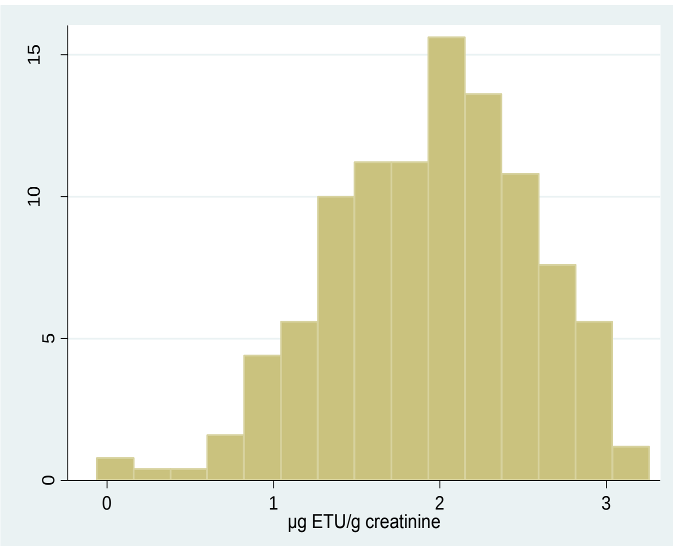
Results

Table 3 presents the characteristics of the children in the study. Boys made up 54% of the participants and the mean age of all children was 9.2 years old (± 0.9). Second and third graders comprised the majority with 4 kids in 1st and 7 kids in 4th grade. All children had detectable ETU levels in the urine samples. The geometric mean (GM) for ETU in the urine samples was 6.96 µg ETU/g creatinine (range= 0.96 – 25.98 µg ETU/g) (Figure 2). The 95% confidence interval (CI) for the GM was 1.75 µg/g – 1.93 µg/g. An extreme outlier value of 110.3 µg ETU/g was reported for one child and eliminated from all statistical analysis.

Table 3. Study Participant Characteristics.

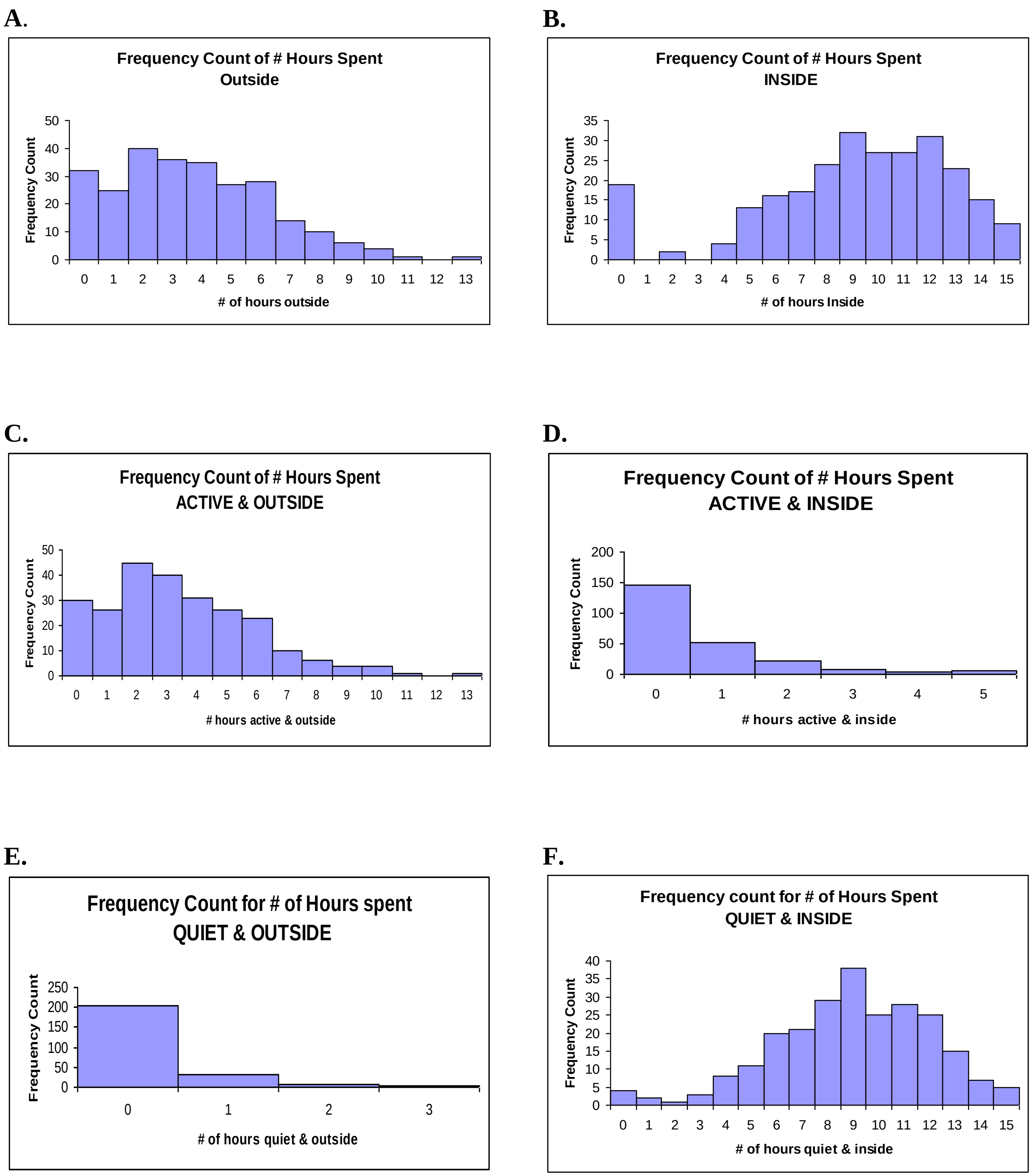
Sex, n (%)	
Boys	20 (54)
Girls	17 (46)
Age, Mean ± SD	9.2 ± 0.9
Grade Level, n (%)	
1st	4 (11)
2nd	14 (38)
3rd	12 (32)
4th	7 (19)

Figure 2. Distribution of all urinary log ETU (µg/g), creatinine adjusted.
Geometric Mean = 6.96 µg ETU/g creatinine; Range = 0.96 – 25.98 µg ETU/g.



In Figure 3, it can be seen that children generally reported spending more hours inside than outside on their activity diaries. The frequency count is the number of responses recorded for the entire study by all children that reported being in a particular microenvironment. This frequency count is not broken down by day of the study. For example, in graph c., there were 45 responses by children reporting that they spent 2 hours in the active and outside microenvironment. The same child could have reported this several times during the period of the study, but that is not distinguished by this figure. In terms of microenvironment, children reported spending more hours quiet and inside as opposed to hours active and outside. The number of hours spent active and inside (d) or quiet and outside (e) were nearly negligible.

Figure 3. Frequency graph # of hours reported in different microenvironments.



In order to validate the children's' responses on their diaries, external observations were made by researchers. There were 7 children whose responses matched our external observations 100% of the time and 4 children whose responses matched our external observations 75% of the time.

Table 4. General study results by day.

Day	Spray	# of kids participating in study	# of surveys collected from kids	# of ETU results
1	Y	35	35	35
2	N	35	35	36
3	Y	37	37	37
4	N	37	37	37
5	Y	37	36	36
6	Y	37	35	34
7	N	37	36	37

Table 5. Urinary ETU levels (μg ETU/g creatinine) according to activity and location options for each day of study.

μg ETU/g creatinine, GM of median

Day	1			2			3			4			5			6			7	
Spray	Y			N			Y			N			Y			Y			N	
Outside	n	GM	95% CI	n	GM	95% CI	n	GM	95% CI	n	GM	95% CI	n	GM	95% CI	n	GM	95% CI	n	GM
<5 hrs	24	8.9	7.1 - 11.4	25	8.6	6.9 - 10.9	29	8.1	6.7 - 10	29	6.2	5 - 7.5	23	6.6	5.3 - 8.3	16	4.7	3.1- 7.1	17	5.8
>=5 hrs	10	8.0	5 - 12.5	10	7.9	4.2 - 14.8	7	6.5	4.7 - 8.9	7	6.0	3.5 - 10.6	12	6.8	4.8 - 9.8	18	7.0	5.4 - 9.1	19	5.7
Active_outside																				
<3 hrs	15	8.6	6.4-11.4	15	9.4	6.6-13.1	21	8.6	6.8-10.9	23	6	4.7-7.7	11	5.3	3.6-8	10	4.7	2.6-8.5	6	6
>=3 hrs	19	8.7	6.3-12.0	20	7.7	5.5-10.8	14	6.8	5.2-8.8	13	6.4	4.9-8.5	25	7.3	6-8.9	23	6.4	5-8.3	30	5.9
Quiet_Outside																				
<=1 hr	28	8.9	7.1 - 11.2	32	8.5	6.7 - 10.8	33	7.7	6.4 - 9.2	33	6	5 - 7.2	34	6.8	5.7 - 8	30	5.9	4.5 -7.4	33	5.7
>=2 hrs	4	6.5	2.8 - 15.3	1	6.8	NA	1	12	NA	2	9.6	NA	1	2.5	NA	1	6	NA	2	6.1
Inside																				
<=8 hrs	21	9.3	7.3 - 12.0	15	8.2	5.3 - 12.6	9	7.9	5.7 - 10.9	7	9.3	6 - 14.4	9	8.8	5.1 -15	14	5.3	3.4 - 8.3	14	5.8
>8 hrs	14	7.7	5.2 - 11.4	21	8.6	6.7 - 11.2	28	7.8	6.3 - 9.5	30	5.6	4.7 - 6.8	27	6.0	5.1 - 7.4	20	6.2	4.7 - 8.2	33	4.8
>=5 hrs	10	8.0	5 - 12.5	10	7.9	4.2 - 14.8	7	6.5	4.7 - 8.9	7	6.0	3.5 - 10.6	12	6.8	4.8 - 9.8	18	7.0	5.4 - 9.1	19	5.7
Active_Inside																				
<=1 hr	22	7.8	5.9 - 10.2	31	7.8	6.1 - 10	29	7.3	6 - 8.8	30	5.9	4.7 - 7.2	30	6.2	5.1 -7.6	26	6.4	4.8 - 8.4	29	5.8
>=2 hrs	9	8.9	5.8 - 13.6	3	12	5.8 - 25.8	7	10	6.4 - 15.6	6	7.9	6.1 - 10.4	6	8.9	6.2 - 12.8	6	4.2	2.2 - 7.9	5	5.6
Quiet_Inside																				
<8 hrs	16	8.4	6.2 - 11.3	11	8.1	4.5 - 14.1	5	8.3	5.1 - 13.4	6	8.7	4.5 - 17	5	5.7	2 - 16.0	9	5.2	3 - 8.8	15	5.7
>=8 hrs	15	7.8	5.5 - 11.1	23	8.2	6.4 - 10.5	31	7.7	6.3 - 9.3	30	5.7	4.8 - 6.9	30	6.7	5.5 - 8.1	23	6.2	4.6 - 8.4	20	5.9

Table 4 presents information on general results of the study for each day of the study whether pesticide spraying occurred, the number of participants who provided a urine specimen, and the number who completed diaries. Table 5 presents geometric means of ETU concentrations and 95% confidence intervals are reported for above and below the median number of hours outside, active and outside, quiet and inside, inside, active and inside, quiet and inside. The two categories highlighted in gray are active and outside and quiet and inside because those were the categories that received the most responses. From the table, it appears that the highest levels of ETU occurred on the first and second days (GM = 9.3 µg/g and 9.4 µg/g) respectively, most likely influenced by spraying on Day 1. When looking at the highlighted rows of active and outside and quiet and inside, the GMs are similar for most days except for Day 4. The GMs from quiet and inside should be considered carefully because there are few counts for this option. On other days, GM values ranged from about 4 – 6 µg/g creatinine.

Several multivariate linear regression models are presented in Table 6. Model 1 shows that on days with no pesticide spraying, children that are active and outside 7 or more hours do not have higher levels of ETU compared to children that are active and outside less than 7 hours ($p = 0.09$). However, there is a possibility that the levels of ETU for both groups of children might be similar on days where no spraying occurred if the study population was larger. Children who are active and outside 7 or more hours on spray days have higher levels of ETU than children who are active and outside 7 or more hours on days where spraying did not occur ($p = 0.007$). But on days where spraying occurred, there was no significance in mean difference of ETU levels among children active and outside 7 or more hours or children that were outside and active less than 7 .hours. Model 4 shows that age and gender are not significant predictors of ETU levels in children. The interaction term explored in Model 4 shows that there was significant evidence of interaction between being active & outside for 7 or more hours and ETU on either spay or non-spray days ($p = 0.008$).

Table 6. Multivariate Linear Regression Analysis

	# of hrs Active & Outside	Occurrence of Pesticide Spraying				
Model 1						
Baseline	**<7**	**no spray**	Estimate	P value	95% Confidence Interval	
Parameters	≥7	no spray	-0.38	0.09	-0.83	0.07
	≥7	spray	0.29	0.146	-0.11	0.68
	<7	spray	0.01	0.825	-0.09	0.11
Model 2						
Baseline	**≥7**	**no spray**	Estimate	P value	95% Confidence Interval	
Parameters	≥7	spray	0.67	0.007	0.19	1.15
	<7	spray	0.39	0.059	-0.02	0.8
	≥7	no spray	0.38	0.093	-0.07	0.83
Model 3						
Baseline	**≥7**	**spray**	Estimate	P value	95% Confidence Interval	
Parameters	<7	spray	-0.46	0.1	-1.0	0.10
	≥7	no spray	-0.85	0.008	-1.4	-0.24
	<7	no spray	-0.47	0.12	-1.1	0.13

Model 4				P		
	Outcome	ETU (log transformed)	Estimate	value	95% Confidence Intervals	
	Parameters	Age (years)	-0.12	0.28	-0.34	0.1
		Gender (boy=0, girl=1)	-0.05	0.78	-0.4	0.31
		Spray (no=0, yes=1)	0.01	0.81	-0.08	0.11
		Active & outside[a]	-0.4	0.06	-0.81	0.01
		Active & outside & spray[b]	0.65	0.008	0.18	1.13

a. Active & outside ≥7 hours = 1, <7 hours = 0.

b. Interaction term

Discussion

There are no formal studies in children regarding ETU detection, but from this study; we can see that active children on average have 1.7 times higher levels of ETU in their morning urine after days where spraying occurred as compared to non spraying days. There were no significant differences observed between boys and girls. The detected levels of ETU in children are as high as the ETU levels reported in environmental epidemiology studies in adults (Table 1). From Table 1, we can see that the detectable ranges of ETU levels are lower in the general population than in the occupational populations, but ETU levels vary significantly based on the type of occupational work. Children have levels of ETU that are lower than pesticide applicators, higher than the general population and similar levels to agriculture workers that sometimes apply pesticides. When comparing the ETU values of this 2008 study in Costa Rica to studies in the literature, the results of this study are concerning because the children in the Costa Rican community do not work in the fields and their levels of ETU are almost as high as occupational workers in the literature reported (Table 1). Other than our study, only one study reported 100% detection of ETU levels in vineyard workers (Colosio et al. 2002).

One strength of this study is that we were able to take repeat measurements in each child to examine how ETU levels vary between different children and within the same child on different days. The high ETU levels are likely to be due the lack of a barrier zone between the banana plantations, community homes, and schools. As a result of this community environment, children can become literally sprayed with pesticides. And due to the hot climate, there are also no adequate coverings on home or school windows, which can increase the community's exposure to mancozeb from drift due to aerial spraying. In addition, although it is forbidden, children reported playing in the banana fields without permission, likewise increasing their exposure to ETU.

The multivariate linear regression model suggests that being active and outside on a spray day is a good predictor of high urinary ETU levels. Children who were active and outside on days where spraying did not occur had, on average 1.7 times lower levels of ETU detected in the urine analysis. These results indicate that spraying contributes to higher levels of ETU detected in urine, especially when children are active and outside for seven or more hours on spray days. All analyses were completed by clustering the same child together to account for intra-variability differences.

When the activity and location variables are broken down by medians of each day, it can be seen that the first and second days of the study yielded the highest ETU values. It makes sense for the first day to have the highest ETU levels because heavy pesticide application was observed over the school and corresponding field. It is possible to say that ETU values on the second day of the study are high because of the second exposure that resulted from the contaminated environment.

There are several positive aspects of the visual time activity diary. The physical design of the survey was well accepted by the participants in terms of convenience because many children were seen wearing the diary when they were playing and of about 260 surveys distributed over the 7 day study period, only four surveys were lost. A diary with fewer options and simple instructions was the better choice for the children than a diary with many activity options. Clear, simple pictures like using a “moon” to represent the evening aided the children’s understanding better than using a word like “evening” on the diary. The visual aspect of the diary significantly helped younger children in the study that could not read well. Even though we used a simple diary structure, almost all children had to be individually trained in addition to the initial group trainings to make sure they were completing the dairy accurately. Children that struggled with completion of the diary in the beginning improved after receiving helped from their parents and or older siblings. The biggest issue faced by the children was how to determine what it meant by “majority of the hour”. We explained that if they did one particular activity for more than 40 minutes that should be the activity to mark down on their diaries. The children were especially motivated to complete the diaries when we offered a secret prize for children that did the best job, but in reality all children received prizes at the end of the study. All children are capable of completing this visual time activity diary, because their age or sex is not a factor in their success, but rather how strongly they can be motivated to participate.

A possible limitation of the visual time activity diary as a tool is how accurate and valid the responses are. To validate the children’s responses, we conducted detailed observations of the children to make note of their locations and later compared our observations of their locations with their responses. There were three limitations to this part of the study. First, we did not devise a structured observation method assuring that all children would be observed an equal number of times. Instead, we observed children whenever possible, which also means that some children were observed more times than other children. Second, only the location (inside or outside) of the children could be observed and not the activity they were doing. This was especially important when they were inside because we did not want to gather observations by asking what they were doing inside, which would disturb the children's normal activities. Third, children reported their activity for the "majority of the hour" but our observations only allowed us to observe them for few minutes each time. Therefore, if our observations do not match their responses, it does not necessarily indicate that their response was incorrect. It may just mean that our observation was not the activity or location in which they had been engaged in for the majority of the hour.

In summary, we report that there is evidence to suggest that children who are active and outside for 7 or more hours have higher levels of ETU on days where spraying occurred compared to children with the same activity levels on days where no spraying occurred. We also pilot-tested a visual time activity diary for children ages 7 – 9 years old in Costa Rica and found that the children were successful in using this tool as evidenced by the high completion rate. Accuracy of the responses, however, needs to be further validated. If this tool were to be adapted for children on other banana villages in Costa Rica, the chances of success should also be relatively high, pending adequate training of the children and support from other family members. However, the physical form of the tool and the contents of the survey might have to be adapted for each agricultural location, special needs to each new group of children, and for the researchers' interests. Although our use of this tool proved to be very successful in terms of response rate, further validation of the accuracy of children's responses is needed through systematic external observations, especially when working with a new group of children. External observations will still have to be conducted in order to validate the children's responses, but this method of validation will most likely be subject to the same limitations as in our study.

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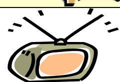
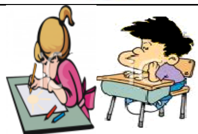
Actividades

Instrucciones:

- En cada hora escriba el número de la actividad que realizó en la mayor parte del tiempo.
- Marque con un círculo el lugar de la actividad que realizó.
- En la hora de los recreos escriba la actividad y marque el lugar.
- En la noche, escriba la cantidad de bananos que comió durante el día.

1		**Durmiendo**
2		**Viendo television**
3		**Comiendo**
4		**Tranquilo (estudiando, leyendo, dibujando)**
5		**Activo (saltando, jugando deportes)**
6		**Otra**

MAÑANA

ESCUELA	HORA	ACTIVIDAD	LUGAR: Casa	Aula	Afuera	Bananal
	6am 7am					
	7am 8am					
RECREO	8am 9am					
	9am 10am					
RECREO	10am 11am					
	11am 12pm					

TARDE

ESCUELA	HORA	ACTIVIDAD	LUGAR: Casa	Aula	Afuera	Bananal
	12pm 1pm					
	1pm 2pm					
RECREO	2pm 3pm					
	3pm 4pm					
RECREO	4pm 5pm					
	5pm 6pm					

NOCHE

HORA	ACTIVIDAD	LUGAR: Casa	Afuera	Bananal	¿Cuantós bananos comió hoy? 
6pm 7pm					
7pm 8pm					
8pm 9pm					
9pm 10pm					
10pm 11pm					
11pm 12am					

Actividades

Instrucciones:

1. En cada hora escriba el número de la actividad que realizó en la mayor parte del tiempo.
2. Marque con un círculo el lugar de la actividad que realizó.
3. En los recreos, marque el número y lugar de la actividad.

1		**Quieto** Durmiendo Viendo television Comiendo Estudiando Leyendo Dibujando
2		**Activo** Jugando deportes Corriendo Brincando Barreando Nadando

MAÑANA

Hora	Actividad		LUGAR		
			Adentro	Afuera	Bananal
7am	1	2			
8am	1	2			
9am	1	2			
10am	1	2			
11am	1	2			
12am	1	2			
Recreo	1	2			
Recreo	1	2			

TARDE

Hora	Actividad		LUGAR		
			Adentro	Afuera	Bananal
1pm	1	2			
2pm	1	2			
3pm	1	2			
4pm	1	2			
Recreo	1	2			
Recreo	1	2			

NOCHE

Hora	Actividad		LUGAR		
			Adentro	Afuera	Bananal
5pm	1	2			
6pm	1	2			
7pm	1	2			
8pm	1	2			
9pm	1	2			

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