Risk characterization of children exposed to aerial sprayings of Mancozeb and ETU

- A case study in a banana village, Costa Rica

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This study is an additive to a previous study performed by van Wendel de Joode et al. (2008) and has been executed as part of a greater collaboration project between Occupational and Environmental Medicine, Lund University and Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacionál, Costa Rica.

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Titel: Riskkarakterisering för barn som är exponerade av Mankozeb och ETU - En fallstudie i en bananby, Costa Rica

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Abstract
Aerial spraying is common procedure at banana plantations in Costa Rica even though the plantations often are situated very close to villages. The fungicide Mancozeb, which is metabolized into the more toxic substance ETU, is one of the pesticides used in aerial sprayings. This thesis has evaluated the exposure of ETU to children living in one of these villages and compared that exposure to different reference doses, of which one was set with a probabilistic approach in this thesis. The exposure doses exceed some of the reference doses and all children have an unacceptable high cancer risk. The main factor that induces high exposure appears to be aerial sprayings close to the village, where Mancozeb and ETU drift into the village. Proposed actions to reduce exposure should be taken at different levels, for example can legislation, timing of aerial spraying activities, education of local health representatives and the searching for alternatives be parallel treatment processes.

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Summary

This study was executed as a master thesis for The Master of Science in Risk Management and Safety Engineering Program at the Department of Fire Safety Engineering and Systems Safety. The study was performed at the department of Occupational and Environmental Medicine, Lund University in collaboration with Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacionál, Costa Rica. The study is an additive to a previous study performed by van Wendel de Joode et al. (2008) regarding children's exposure to several pesticides, in banana villages in Costa Rica. That study has not yet been published but its result was presented at the EPICOH/NEUREOH 2008 conference, Heredia, Costa Rica.

Frequent use of pesticides in banana plantations, which includes weekly aerial applications of fungicides, is a common procedure in Costa Rica. One of these fungicides is Mancozeb, which has low acute toxicity but can cause adverse effects when the exposure is prolonged. The present study was conducted in a village which is surrounded by banana plantations, where Mancozeb is applied by light aircraft or helicopter on several days of the week. The major metabolic product of Mancozeb is Ethylenethiourea (ETU) which is considered to be more toxic than Mancozeb and have induced a wide spectrum of anomalies in many test animals. The purpose of this thesis is to characterize the risk originating from the ETU exposure to children living in this village. The children in the village are also exposed to many other pesticides and chemicals, however any synergistic, additive or antagonistic effects between these substances have not been further investigated.

A dose-response model was constructed in order to derive a reference dose (Rfd) for children's exposure to ETU. Three other reference doses for ETU from U.S EPA (2005), FAO/WHO (1993) and IPCS (1993) were also used, as well as a cancer potency factor derived by U.S EPA for calculating the cancer risk. The children's exposure in the village was estimated by measuring their urinary ETU levels during an eight day long field study and converting the levels in to doses with help from the results from a human oral exposure experiment. The urine was collected for seven consecutive days, observations were conducted regarding spraying activities in relation to where the children lived or spent their days and questionnaires were used to get information about the parents' working situations. The chemical analysis was performed at the *Division of Occupational and Environmental Medicine*, Lund University. A statistical analysis to assess important factors, among those that were observed, leading to high exposure was performed. Subsequently an integration of the results from the dose-response and the exposure assessments was made in order to characterize the risk for the children. Also an uncertainty analysis was performed regarding the assessments.

The results of the integration of the assessments showed that according to the reference dose derived in this study, the reference dose of U.S. EPA and the calculated cancer risk, the risk in the village is unacceptably high regarding the children's exposure to ETU. Spraying close to the village appears to induce high exposure. The results also indicate that fathers who work in the plantation to some extent bring exposure home.

A discussion regarding risk treatment and risk communication was executed and with the regard to that discussion and the result from the integration of the assessments the following conclusions could be made:

- The risk for the children regarding their exposure to ETU is unacceptably high.

- There seems to be a more or less constant exposure to ETU.
- There are reasons to believe that ETU can cause various adverse health effects in the children from a long term perspective.
- The result suggests that Mancozeb and ETU drift into the village and that ETU stays in the environment for a few days and causes exposure to the children
- Our believe is that if the sprayings would occur further away from the village the exposure for the children would decrease the risk
- Actions to reduce the risk should be taken at different levels, for example can legislation, timing of aerial spraying activities, education of local health representatives and the searching for alternatives be parallel treatment processes

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This thesis could not have been executed without the help of several people, both in Sweden and in Costa Rica. In this section some of the people who have helped us will be presented.

First of all we would like to thank the children and their parents in the banana village in Costa Rica, for without their cooperation and hospitableness this study would not have been possible to perform. We must also give out a special thanks to Leonél Córdoba Gambóa for helping us with maps and support during the field study. We would also like to thank Karen Phung for getting up before dawn to help us with our spraying observations. Doña Irma, Doña Paula and Doña Yolanda deserve an extra thank you for the delicate food and all the practical help during the field study. All the people at the IRET office and laboratory should be addressed a big thank you for their hospitableness, helpful advises and for making us feel welcome and a part of the crew. For improving our poor Spanish and surfing knowledge we owe Augusto Cano a thank you as well.

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1 Introduction

This study was executed as a master thesis for The Master of Science in Risk Management and Safety Engineering Program at the Department of Fire Safety Engineering and Systems Safety in a collaboration with Occupational and Environmental Medicine, Lund university and Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacionál, Costa Rica. The study is an additive to a previous study performed by van Wendel de Joode et al. (2008) regarding children's exposure to several pesticides, in banana villages in Costa Rica. That study has not yet been published but its result was presented at the EPICOH/NEUREOH 2008 conference, Heredia, Costa Rica.

Worldwide, Costa Rica is one of the four leading banana exporting countries (www.unctad.org) and bananas have been produced and exported commercially by multinational companies since 1880 (www.fao.org). Bananas are the single largest agricultural export and generated almost 500 million dollars in export revenues, 2001 (www.fao.org). Area used for banana cultivation in Costa Rica has almost duplicated between 1977 and 2002 (de la Cruz et al., 2004 p. 34).

Frequent use of pesticides in banana plantations for protection of crops is a common procedure in Central America (Wesseling, 1997 p. 21). It includes weekly applications of fungicides, by light aircrafts or helicopters, ground application of nematocides and herbicides, and the use of insecticide impregnated bags (Wesseling, 1997 p. 21). These pesticides possess a serious threat to the environment and to the health of people working at the plantations and to those living in the villages surrounded by the plantations (Humbert et al., 2007).

The present study was carried out in one of these villages, which is situated on the Pacific Coast, close to the Panama border. Further on in this thesis, this village will be referred to as "the banana village". It is a small village that is surrounded by banana plantations. The people are mainly indigenous working immigrants from Panama. All the houses are owned by the banana companies, Chiquita or Del Monte. At least one person in each household is employed by one of these companies.

On the plantations surrounding the banana village the fungicide Mancozeb is frequently applied by airplanes or helicopters. Mancozeb has low acute toxicity and it decomposes quickly (Colosio et al., 2006). The major metabolite product of Mancozeb is Ethylenethiourea (ETU) which is a product of both metabolic and environmental degradation (Colosio et al., 2006). In this study the exposure will be assessed for this metabolite on children living in the village. Experimental studies have detected significant correlations between exposure of ETU and adverse health effects, for example incidence of cancer, especially in the thyroid glands (Graham et al., 1975). Experimental studies also indicate neurological effects (Debbarh et al., 2002; Domico et al., 2006) and furthermore there is a possibility that it can possess reproductive toxicity (Cecconi et al., 2007). However, relatively little is known about the effects of long term exposure of ETU in humans. Also there is very little knowledge of what effects can be expected in exposed children. The children in the village are also exposed to many other pesticides and chemicals, however any synergistic, additive or antagonistic effects between these substances have not been further investigated.

The recent study performed by Van Wendel de Joode et al. (2008), which included this village and others in Talamanca, urine samples were collected and subsequently analyzed for the metabolite

ETU. The study indicated that differences in ETU exposure levels were largest between villages, followed by children and days. The differences between villages indicate that aerial application of Mancozeb correlates with high exposure, whereas exposure is low in villages where there is manual or no application of Mancozeb. A factor that partly explained the differences in exposure levels between children was the frequency that the children entered the banana plantations. Another finding from the study was that urinary ETU levels were higher after aerial spraying days. However, this relation was based on few measurements.

In order to better understand the differences between the exposure of children living in the village, to evaluate the risk correlated to ETU exposure and to find possible solutions to reduce this risk the current study will be performed according to the following task description.

1.1 Task description

1.1.1 Purpose

This thesis will be carried out as a risk management process and the purpose of it is to characterize the risk for the children being exposed to ETU and to discuss strategies how to reduce the risk. No previous study within this field has been conducted with such a large quantity of data. Hopefully new aspects on children's exposure to pesticides, factors inducing high exposure and the mechanism of uptake and excretion of ETU can be enlightened.

1.1.2 Research questions

- How can the risk for children in the banana village exposed to Mancozeb and its metabolite ETU be characterized?
    - To what extent are levels of ETU acceptable in children for long term exposure?
    - Do the levels in the children exceed the reference dose as presented in this report?
    - What factors induce high levels of ETU?
- How do exposure levels relate to aerial spraying applications of Mancozeb?
- Is there a correlation:
    - Between level of exposure and distance to aerial spraying activities?
    - Between levels of exposure in parents and their children?
    - Between levels of exposure in the child and the parents´ task in the banana company?
- Which strategies to reduce health risks, associated with exposure to Mancozeb and ETU, can be proposed and have been proposed by authorities and other stakeholders?

1.1.3 The risk management process

The risk management process as described in this thesis is an integrated process with inspiration from the definitions from ISO/IEC (2002) and risk assessment frameworks according to WHO (2001), NRC (1983) and U.S. EPA (1992a) (*Figure 1*).

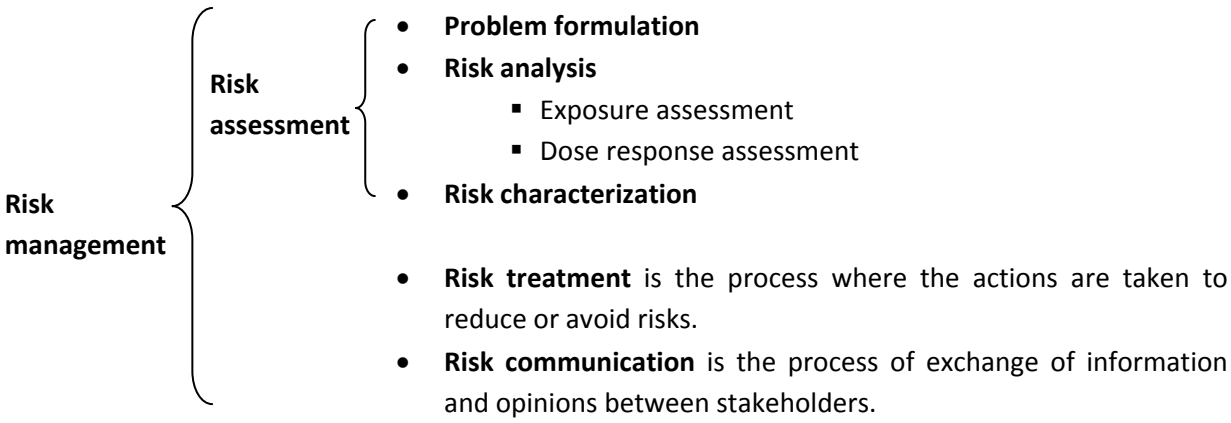

Figure 1. *The risk management process inspired by ISO/IEC (2002), NRC (1983), WHO (2001) and U.S. EPA (1992a)*

In this thesis there will be a focus on the risk assessment process, and the risk treatment and risk communication will be subject for discussion. More details about the risk management process can be found in Chapter 4.

1.2 Restrictions and limitations

The aim of the thesis is to focus on the exposure to ETU, thus any additive, protective or synergic effects of other pesticides or other chemicals will be neglected.

The effects of Mancozeb and ETU will not be monitored in the village and hence not be taken into account in the risk characterization.

The thesis only assesses the risk for the children in the village, thus the risk for the workers or the general public will not be considered.

1.3 Disposition

The **Background (Chapter 2)** will give the basic knowledge about the conditions in the village, pesticide use in Costa Rica and pesticide use on banana plantations. Also, concerned stakeholders will be identified and presented.

In the chapter about **Mancozeb and ETU (Chapter 3),** the toxicological and metabolic aspects of Mancozeb and ETU will be highlighted.

In the **Risk Theory** chapter **(4)** the theoretic aspect of Risk Management, which is used in this thesis, will be clarified.

In the **Methods for Risk Assessment** chapter **(5)**, different methods that can be used in environmental and health risk assessments are presented to show how such an assessment can be performed.

The chapter about **Methods used in this thesis (Chapter 6)** describes the methodic for this particular study.

The chapter of **Results from the risk assessment (Chapter 7)** presents the result and discusses the uncertainties originating from different parts of the process.

There will be a **Discussion (Chapter 8)** on how the result from this thesis can constitute a base for risk reduction strategies and be communicated to concerned stakeholders.

Finally**, Conclusions (Chapter 9)** from the risk assessment and the discussion of risk reduction and risk communication are presented.

2 Background

2.1 The banana village

The banana village is situated in Talamanca County, which is the county with the lowest human developmental index in Costa Rica (Hermida Viallet et al., 2007). It is located near the Pacific Coast, close to the Panama border (*Figure 2*). The climate in the area is humid and warm which make it ideal for banana cultivation.

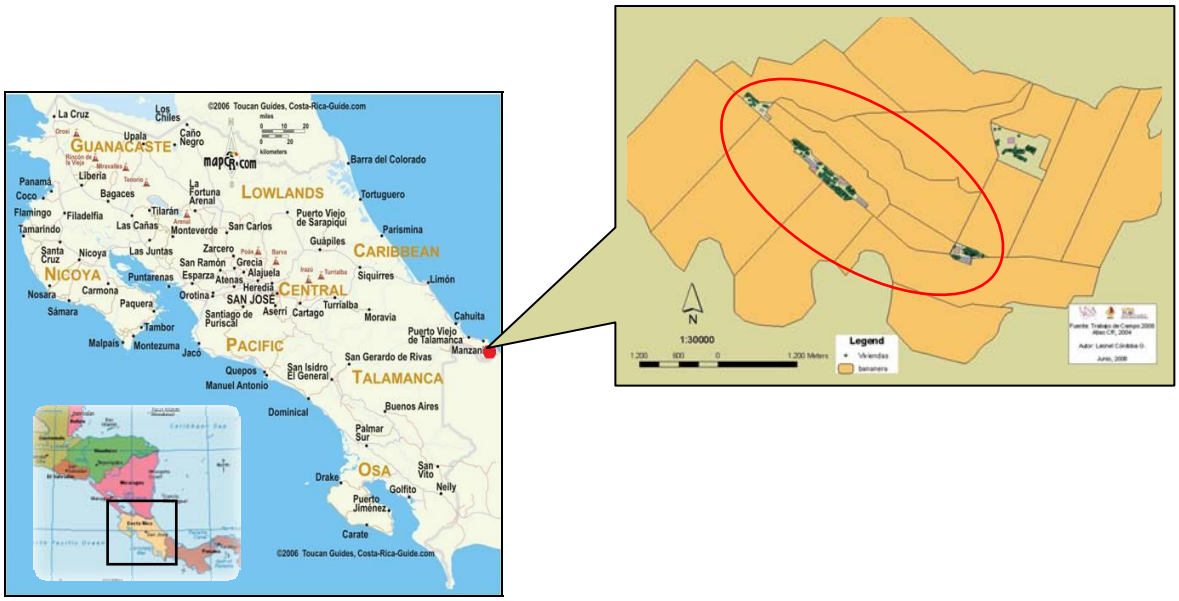

Figure 2. *The location of Costa Rica and the village. The right map is of the village. All the yellow is banana plantations. Central America map: http://schema-root.org, Costa Rica map: www.beachbumparadise.com, Village map: Córdoba Gamboa, (2008).*

The village is surrounded by banana plantations, which are owned by the companies Chiquita (South west of the village) and Del Monte (North east of the village). These companies also own all the houses in the village, which consequently means that at least one member in each household is working for one of the companies. According to Sosa et al. (2006) the major part of the population in the village are adults between 20 and 59 years of age, of whom 80 % are active workers. The remaining 20 % are housewives and do not work outside their home (Sosa et al., 2006). Generally in the study, the father was working in the company and the mother was at home taking care of the children. In some cases, both of the parents or only the mother was working for the companies.

Many of those who live in the village are indigenous people from Panama that are working immigrants (Sosa et al., 2006). There are also immigrants from Nicaragua and native Costa Ricans. According to Sosa et al. (2006) there are social actors in the community that perceive the working conditions in the village as poor. This is because a major part of the population does not possess sufficient labor contracts, which means that they get too low salaries and lack employment security (Sosa et al., 2006). Generally in Costa Rica, workers' organizations are weak, in particular in the agricultural sector, and workers have little or no input into company decisions, including those having to do with pesticide related risks (Wesseling et al., 2001).

There is a primary school in the village where children get education until sixth grade. Those who want to continue studying have to travel for up to half of an hour to do so (Sosa et al., 2006 refer to ASIS Daytonia, 2006). According to Sosa et al. (2006) some adolescences leaves school to work at the plantations to be able to help the family economically.

Even if there is a clinic in the village, many people do not use it. Immigrants that participated in focus groups say that they are not treated well at the clinic (Sosa et al., 2006). Also, the clinic lacks some important equipment like laboratories to be able to respond to the needs in the village. For example, to give birth, one has to travel to the town of Limón (*Figure 2*).

The majority of the houses (*Figure 3*) are in acceptable conditions, according to people at the clinic (Sosa et al., 2006). This is based on quality of sanity facilities, accessibility of drinkable water, electricity, quantity of electrical products etc. Houses that belong to the companies are equipped with toilets and are administrated water through wells owned by the companies (Sosa et al., 2006 refer to ASIS Daytonia, 2006). People in the village do not have their own gardens and they get their food from buying it in the supermarket (Sosa et al., 2006). During the field study, we perceived the houses in the village as simple and small for large families. In some houses, we doubted that there were enough beds for everyone in the family.

Figure 3 *The picture shows some of the houses in the village.*

2.1.1 Aerial spraying in the village

According to Sosa et al. (2006) there are aerial spraying activities between 5 a.m. and 7 a.m. and between 4 p.m. and 5 p.m. (*Figure 4*). Sometimes, there are applications in the middle of the day, depending on wind conditions. This information is supported by a worker in the village who also pointed out that there is no spraying when it is raining. The workers are notified about the spraying schedule, so that they do not enter the plantation. However, the rest of the population is not informed, and there is no fixed schedule. According to Costa Rica law, aerial spraying activities are forbidden within 30 m distance to houses in case of a natural barrier and 100 m without such a barrier (Reglamento para las actividades de la Aviación Agrícola, 2003). However, the law does not regulate growing crops close to the residential zone.

Figure 4. *Spraying over banana plantations in the banana village.*

2.1.2 Conceptions about pesticides in the village

Information about pesticides and protection against them is only provided to the workers by the companies (Sosa et al., 2006). Occasionally they carry on the information to their families. For the moment, there are no institutions that provide information about problems associated with pesticides. In focus group discussions moderated by Sosa et al. (2006) subjects like perceived risk and exposure routes among people in the banana village were raised. Some of the results from that study are presented below.

The people in the banana village think that pesticides are something that is damaging to the health. To them, pesticides are everywhere in the environment. They believe that the chemicals are transported by the air, fall down on the roofs, on the clothes that are hanging to dry, on the skin of people and on everything that is outside. Some people in the focus group expressed their worries about the water they receive in their houses; they think that it might be contaminated with pesticides.

Among the health problems that people in the village associate with pesticides are skin problems and headache. They are aware of the fact that pesticides can cause chronic health problems, but they do not know exactly what kind. There is an uncertainty about the real health effects from pesticides, and some people have started to think that health problems like diarrheas and tuberculosis are associated with pesticide exposure. One of the causes behind this fallacy could be the doctors' tendency to hand out medicine without explaining what causes the illnesses.

2.2 Stakeholders

A stakeholder is usually defined as a person or a group who has a stake or special interest in an issue, policy, company, etcetera (Welp et al. 2006). Stakeholders in the risk management process are members of the society who are concerned about the issues associated with the management and who may be affected by the decisions (WHO, 2001 p. 20). The purpose of including stakeholders in this thesis is that they are the ones that can use the results of the assessment and influence the situation, and also the ones that are affected by it. Welp et al. (2006) stress the need for science to have access to the insights and expertise of different societal actors and incorporate their knowledge bases. Also scientists need to communicate the results of their inquiries in a comprehensible way (Welp et al 2006). Potential stakeholders in a risk management process are representatives of industry, public interest groups, property owners and resource consumers (WHO, 2001 p. 20).

2.2.1 Identification of Stakeholders involved in this project

With help from van Wendel de Joode (Mail communication, November 2008) stakeholders in this risk management process were identified as (in alphabetical order):

- *Aviación Civil*-The civil aviation administration, (www.dgac.go.cr/)
- ***Banana Companies***
- *Camara de insumos aguapecuarios*- an organization consisting of providers of products and technology within the agricultural sector, (www.insumos.cr)
- ***Certification organs***
- ***CORBANA (National Banana Cooperation)***
- *Ministry of Agriculture*

- *Ministry of Environment*
- ***Ministry of Health***
- *Ministry of Production*
- *Ministry of Work*
- *OPS-OMS* -Division of health organization in Costa Rica, (www.cor.ops-oms.org)
- ***People in the village***
- *Pesticide industry.*
- *Teachers*
- ***University***

Stakeholders accentuated in the list are those that are identified as the most important regarding this study and will be described further. This identification is by no means complete but probably includes the most important actors at an overall level. There are also possible stakeholders at a local level that will not be investigated in this thesis.

2.2.2 Functions of the Stakeholders

A short presentation will be given of each stakeholder that is considered to be important for this study. ***People in the village*** have previously been presented and no further information about them is given here.

2.2.2.1 Banana companies

The two banana companies, Bandeco, that is a subsidiary to ***Del Monte*** (www.fao.org) and ***Chiquita*** employ the majority of the population in the region. They strongly influence the socioeconomic conditions in the region and both of the companies favor a system for employing working immigrants (Sosa et al., 2006).

2.2.2.2 Certification organs

All Chiquita's banana farms in Costa Rica are certified by Rainforest Alliance (www.rainforest-alliance.org). One criterion that has to be fulfilled to keep certification is that no substances that are forbidden by US U.S. EPA or the European Union can be used in the farm.

Del Monte plantations in Costa Rica are certified in accordance with Global Gap (formerly known as Eurep GAP), SA-8000, Social Accountability, ISO 9001 (Quality Management Systems), ISO 14000 (Environmental Management Systems, OHSAS 18001 (Occupation Health and Safety) (Del Monte, Mail communication, September 2008). Del Monte states on their website that they do not use products banned by the United States, European Union, or World Health (www.freshdelmonte.com).

A more detailed presentation of the different certifications is found in Appendix A

2.2.2.3 CORBANA

Corbana is a non-governmental entity that controls the banana industry in Costa Rica (Sosa et al., 2006). The shareholders are divided in to three equal parts, consisting of people from the government, national banks and all the banana producers in the country. Their activities are divided into: spreading information to politicians that influence the industry, scientific investigation within agricultural technology, financial support and providing information about the market.

2.2.2.4 Ministry of Health

The ministry of health is responsible for the registration of pesticides and the evaluation of chemical substances entering the country (Sosa et al., 2006). In this way, this institution influences the availability of chemicals that is used in the country.

The division of ministry of health in Talamanca has the objective to protect and promote the health of people living in the county through different strategies. They are responsible for the inspections of the banana plantations and packing facilities, to assure that pesticide application is done correctly and that personal protective equipment is adequate (Sosa et al., 2006). They also provide training and perform health controls of the workers.

2.2.2.5 University

IRET (El Instituto Regional de Estudios en Sustancias Tóxicas) at Universidad Nacionál have experience in occupational health studies within the agricultural sector. They were involved in the previous study in the village as well as this one and are deeply involved in the project. This stakeholder is also the first instance to which the result from the present study will be reported and will be the one that communicate the result to other stakeholders.

2.2.3 Collaboration

The information in this chapter was provided by van Wendel de Joode (Mail communication, November 2008).

A committee was formed with the aim to discuss the children's pesticide exposure and possible health effects related to the banana cultivation and actions to reduce the exposure. This committee consists of representatives from the University, the Banana Companies, the Ministry of Health, Ministry of Environment, CORBANA, OPS-OMS, Aviación Civil and Camara de Insumos Aguapecuarios. The meetings have been focusing on possible actions to take and there has been an agreement that exposure should be reduced. However there have been difficulties in collaborating because of different interests.

A subcommittee was also formed in order to work more efficiently with this subject. This consists of represents from the University, Ministry of Health and CORBANA. There is also a second subcommittee, a regional one, in Talamanca, with representatives of Ministry of Heath, Ministry of Education, Ministry of Environment, and the Municipality. However, there has been a change of personnel in this group which has slowed down the process. More results from these collaborations are discussed in Chapter 8 about risk treatment and risk communication.

2.3 Pesticides

Pesticides are designed to interfere with a variety of biological targets in living organisms (Wesseling, 1997 p. 11). The term pesticide usually refers to insecticides, nematocides, herbicides, fungicides, rodenticides, growth regulating agents, and fruit thinning agents (WHO/UNEP, 1990 p. 11). As living organisms share many biological systems, the pesticide actions commonly affect non-target species as well, including humans (Hayes & Laws, 1991 vol 1, p 9).

2.3.1 Pesticide use in Costa Rica

Agriculture represents an important economic activity in Costa Rica (Wesseling et al., 2001). Like in other developing countries, agriculture in Costa Rica depends on high chemical input, associated with

excessive use of pesticides (Wesseling et al., 2001). An annual average of 2.5 kg of active ingredients per inhabitant was estimated for 1996, which can be compared to the rate in Netherlands which is 0.7 kg/inhabitant and represents a typical European rate (Chaverri & Blanco, 2002). Even if conditions for workers are getting better, and some restrictions regarding pesticide use are getting through, various pesticides are still in use that is banned or restricted in other countries. Mancozeb for example, is restricted regarding both application rate and pre-harvest application in Sweden and in the United States of America (www.kemi.se; U.S. EPA, 2005 pp. 2-3) Mancozeb is one of the pesticides imported in highest volumes to Costa Rica (de la Cruz et al., 2004 p. 22).

According to Sosa et al. (2006), no action program exists in Costa Rica that focuses on long term exposure to pesticides or exposure to children.

2.3.2 Pesticide use in banana cultivation

Banana cultivation is one of the crops that use the largest amount of pesticides in Costa Rica, both considering per ha and in total (de la Cruz et al., 2004 pp. 38-42). The plant (*Figure 5*) is a perennial crop that grow quickly and can be harvested all year round (www.fao.org). Plantations are based on monocultures and the size of them normally ranges 150-200 ha (Vargas, 2006). Humid, tropical lowland climate with high temperatures and large amounts of rainfall, in combination with monoculture plantations, lead to severe attacks of pests and diseases (Vargas, 2006). There are many types of pesticides that are applied on banana plantations to fight these pests. An organophosphate nematocide is applied two or three times a year in cycles which last four to eight months (Wesseling, 1997 p. 21). As a shelter from insects, plastic bags with the organophosphate insecticide Chlorpyrifos are placed around the fruit in the field as a barrier (Wesseling, 1997 p. 21). The herbicides Paraquat and Glyphosate are sprayed with back-packs the year around (Wesseling, 1997 p. 21). Extensive use of pesticides is also common after harvest, i.e. when cleaning and packing the bananas (Wesseling, 1997 p. 21).

Figure 5. *A banana plant*

One of the most damaging occurring diseases is the Black Sigatoka leaf spot disease (www.fao.org). It affects the growth and productivity of plants and is the main reason why fruit is rejected by exporters (www.fao.org). The fungus decreases photosynthesis, reduces fruit size and induces a premature maturation (www.fao.org). This disease requires weekly applications of fungicide mixtures containing amongst others Mancozeb (Vargas, 2006). It is sprayed up to 47 times all year around by aeroplane or helicopter (Wesseling, 1997 p. 21). The cost of controlling the disease in large plantations is about $1,000 per hectare, but it is higher in smaller plantations that cannot apply by air (www.fao.org).

3 The fungicide Mancozeb and its metabolite ETU

As mentioned in the introduction, this thesis is focusing on the health risks concerning ETU. This is mainly because it is only the exposure of ETU that is assessed and can be estimated. It is also partly because many of the health risk concerns for Mancozeb are driven by the risk from ETU (U.S. EPA, 2005 p. 5). However, even if it is difficult to estimate the exposure to Mancozeb itself, it is a fact that the children to some extent are exposed to Mancozeb as well as ETU. Because of this a summary of the properties of both ETU and Mancozeb are presented below.

3.1 Mancozeb

Mancozeb $(C_4H_6MnN_2S_4)_a(Zn)_y$ (*Figure 6*) is a fungicide that belongs to the ethylene-bis-dithiocarbamate family (EBDC) (Belpoggi et al., 2002). Mancozeb and similar substances were introduced on the pesticide market as early as 1944 and today Mancozeb is one of the most extensively used fungicides in the world and is used on various crops (Belpoggi et al., 2002). This is partly due to its efficacy against a broad spectrum of fungi and their associated plant diseases (Houeto et al., 1995) and partly due to its low acute toxicology and low environmental persistence (Colosio et al., 2006).

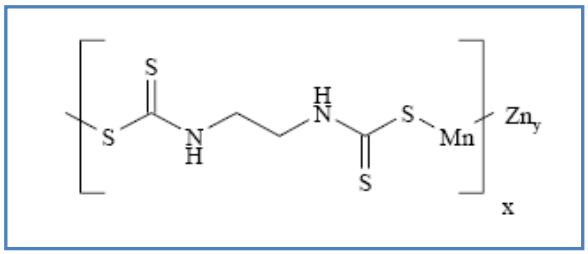

Figure 6. *Chemical structure of Mancozeb (U.S. EPA, 2005 p. 4)*

Mancozeb can be applied with light aircraft and helicopter or ground equipment, such as groundboom and airblast sprayers (U.S. EPA, 2005 p. 6) and it acts by enzyme activity inhibition (Belpoggi et al., 2002). Mancozeb does not occur as a natural product (Belpoggi et al., 2002). It has legible vapor pressure and low potential to volatilize into the environment (U.S. EPA, 2005 p.49). It can be found associated with air-borne particles or as spray drift, it has low solubility in water but hydrolyzes quickly over a wide range of pH, it degrades with heat, and upon exposure to moisture and air (U.S. EPA, 2005 p. 53). The short lifetime in water suggests that there is a low risk of contamination of ground water (U.S. EPA, 2005 p. 22).

3.2 ETU

Ethylenethiourea (ETU) ($C_3H_6N_2S$) (*Figure 7*) represents the main degradation product of the EBDCs (Sottani et al., 2003) and does not occur as a natural product (Arbetslivsinstitutet, 2001 p. 2). ETU represents not only a metabolic compound, but it is also a product from environmental degradation and is formed when cooking food with residues of Mancozeb and the other EBDC fungicides (U.S. EPA, 2005 4). Further on, it is present as an impurity in several EBDCs formulations (Colosio et al. (2006) refer to Bontoyan et al., 1972; Lindh et al. 2008; Kurttio et al. 1990). Lindh et al. (2008) found 4.5 % ETU in a commercial Mancozeb product. Apart from being a metabolic product of the EBDCs it is used in the rubber industry for vulcanizing (Arbetslivsinstitutet, 2001 p. 2).

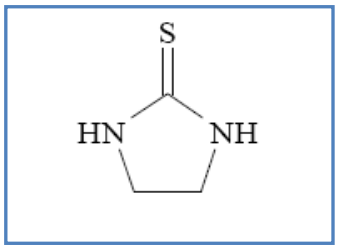

Figure 7. *Chemical Structure of ETU (U.S. EPA, 2005 p. 5).*

ETU is a crystalline solid that in contrast to Mancozeb is soluble in water (U.S. EPA, 2005 p. 5). Due to its persistence against hydrolyzing and high solubility there is a risk that ETU can contaminate ground

water, especially when raining after application (www.kemi.se). The risk of bioaccumulation is low, both for Mancozeb and ETU (www.kemi.se).

3.3 Uptake and Metabolization

According to Steenland et al. (1997) Mancozeb is primarily absorbed dermaly and metabolized to ETU. This is supported by an occupational study by Colosio et al. (2002), where a correlation was found between skin exposure and urinary ETU. Kemikalieinspektionen (www.kemi.se) also reports about animal studies that have demonstrated a dermal uptake of Mancozeb. WHO/IPCS (a) (www.inchem.org) however expresses that metal-complexed alkylene bisdithiocarbamates, of which Mancozeb belongs, are poorly absorbed through the skin. Some goes even further suggesting that Mancozeb is not absorbed through the skin at all (Littorin, Mail communication, November 2008).

WHO/IPCS (a) (www.inchem.org) also stress that Mancozeb would be poorly absorbed through the GI-tract. Kemikalieinspektionen (www.kemi.se) on the other hand emphasize that approximately half of orally distributed Mancozeb is absorbed through the GI-tract. Houeto et al. (1995) suggest as well that Mancozeb is in fact absorbed through the GI-tract.

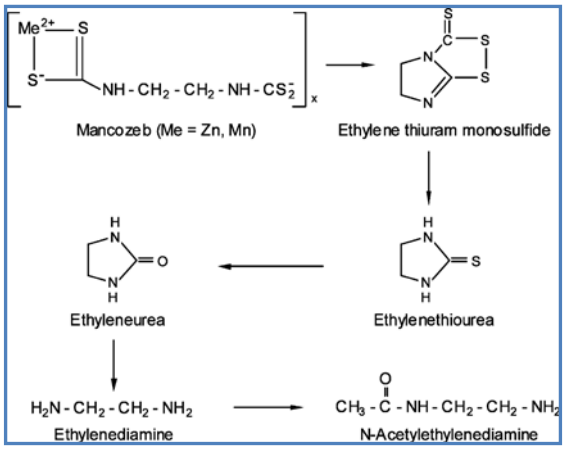

Figure 8. *Metabolism of Mancozeb in mammals (Colosio et al., 2002)*

As can be noted above, how Mancozeb is absorbed is not very clear and different researchers have different conceptions about the issue. However regarding ETU both Houeto et al. (1995) and Lindh et al. (2008) believe that it is absorbed in the GI-tract. This is also held for true by a study presented by FAO/WHO (www.inchem.org), which also describes a dermal uptake for guinea pigs. Dermal uptake of ETU in animal studies is confirmed by Kemikalie-inspektionen (www.kemi.se).

The metabolic decomposition of Mancozeb in mammals is complex and results in the formation of various metabolites, of which ETU is the most extensive and most important from a toxicology perspective (*Figure 8*) (Houeto et al., 1995). Aprea et al. (1997) confirm that residues of ETU are specific for EBDCs and for ETU itself.

> ***Biomarkers***
>
> *A biomarker is a biological molecule found in blood or other body fluids, that is a sign of a normal or abnormal process (www.cancer.gov).*
>
> *The strengths of using biomarkers are that they demonstrate that exposure to and absorption of the chemical has truly taken place (U.S. EPA b, 1992). Also a biomarker reflects the complete uptake (Hoet & Haufroid, 1997).*
>
> *A difficulty with using biomarkers is that it can be hard to relate the internal level to a quantitative dose or exposure (U.S. EPA b, 1992).*

It has been demonstrated that urinary excretion of ETU is a suitable biomarker for exposure to EBDCs (Sottani et al., 2003; Lindh et al., 2008). However as ETU also is present as an impurity in EBDCs solutions it is difficult to tell whether the urinary ETU-levels come from Mancozeb which is metabolized to ETU or ETU itself. In a human study by Lindh et al. (2008), where Mancozeb was ingested, it could not be concluded if Mancozeb was absorbed in the GI-tract. Between 69 % and 82 % of the ETU impurities in the Mancozeb product were found in the urine after

104 hours post exposure. If the Mancozeb was thought to be absorbed and converted to ETU, 14 % of the Mancozeb was recovered after 104 hours. Whether or not the correlation between the dermal exposure and ETU-levels in the occupational study by Colosio et al. (2002) could depend on impurities of ETU in the Mancozeb solution rather than Mancozeb itself can only be subject for speculation.

ETU is distributed to various organs, but does not accumulate since it is eliminated quickly via urine (Cecconi et al., 2007). In a voluntary study on humans the elimination half-life of ETU was estimated to be in the range 19-23 h (Lindh et al., 2008). Those results are comparable with attempts to estimate elimination half-lives in urine after occupational exposure to Mancozeb and other EBDCs where half-life of ETU elimination was estimated to about 100 h (Kurttio et al., 1990). Kurttio et al. (1990) explain the long elimination half-life with slow dermal penetration. Lindh et al. (2008) stress that it is very possible that the half-lives depend on the exposure route. Lindh, (Personal communication, November 2008) proposes that exposure through lungs and through the GI/tract probably leads to a higher uptake rate than dermal exposure.

The excretion rate can also be critical for the toxicity of the substance. For example, because of different metabolic pathways and enzymes, mice excrete ETU faster than rats (Lewerenz & Plass, 1984). According to Lewerenz and Plass (1984) this might explain the difference in acute toxicity where mice show much higher resistance towards ETU than rats.

3.4 Occupational exposure and exposure of the general public

It seems as if occupational studies are mainly focusing on two exposure routes, namely the skin and the respiratory tract (Kurttio et al., 1990; Jablonická et al., 1989; Aprea et al., 1998). While the GI-tract is considered to be the main exposure route for the general population (U.S. EPA, 2005 p. 20; Colosio et al., 2006)

Only a few occupational studies have been conducted for exposure to EBDCs and ETU where ETU levels have been measured in the urine. Steenland et al. (1997) performed one study on backpack sprayers and landowners in a study in Mexico. In that study it was found that backpack applicators had a higher mean than the landowners (*Table 1*). However 34 % of the applicators had levels below the limit of detection while 50 % amongst the landowners. Another study, which was performed for banana plantation workers in the Philippines, showed that directly exposed workers had higher ETU levels than the indirectly exposed workers (Panganiban et al., 2004). Yet another study which indicates occupational exposure is a study by Colosio et al (2002). In this study it was found that urinary levels of ETU from vineyard workers were higher at the end of the working day than at the start. A fourth study has been conducted by Kurttio et al. (1990) on potato farm workers during application of EBDCs. In this study the exposure through lungs and skin was measured and urinary levels of ETU was detected. The individual differences for the urinary levels of ETU were large, although a conclusion made from the study was that approximately 1-10 % of ETU on clothes reached the skin. No comparisons were made of the urinary levels between more and less exposed workers.

See table 1 for numbers from the different studies. The units for the ETU levels in these studies are all different and it is hence not possible to compare the studies with each other. What can be seen though is that in all studies the people who would be expected to have a higher exposure also show

higher levels of ETU in the urine. The levels in the study by Panganiban et al. (2004) are consistently much higher than in the other studies.

Table 1. *Occupational exposure, the three different studies mentioned in the text above and the numbers for the different ETU-levels. The units are different in the different studies and can only be compared within a study.*

Study	Studied subjects	More exposed	Less exposed
Steenland et al (1997)	Landowners and backpack sprayers in Mexico,	Backpack sprayers: 58 ± 26 ng/ml	Landowners: 12 ± 3 ng/ml in
Panganiban et al. (2004)	Banana plantation workers in the Philippines	Directly exposed: 378,34 ± 50,11 ng/ml	Indirectly exposed: 267,16 ± 69,9 ng/ml.
Colosio et al. (2002)	Vineyard workers in Italy	Before work: 0,5 µg/g creatinine Range: <0.5-3.4 µg/g creatinine	After work: 2,5 µg/g creatinine Range: <0.5-95.2 µg/g creatinine
Kurttio et al. (1990)	Potato farm workers	Post exposure, 24 h: 0,1-2,5 µg/mmol creatinine	

As for exposure to the general population two Italian studies have been conducted on that subject. Colosio et al. (2006) estimated the mean ETU in urine in the Italian general population (0.6–0.8 µg/g creatinine). Aprea et al. (1996) performed another study where they measured the urinary ETU levels of people living in cities (mean: 2.7 µg/L) and compared those to ETU-levels of people living on the countryside (mean: 9.1 µg/L). In this study it was also found that people living in areas where there had been aerial spraying of Mancozeb had higher levels in their urine. Their conclusion was that these people might harvest the crop before recommended date and therefore were more exposed.

In the previous study in the banana village, which also measured urinary levels of ETU in children, the mean and median of ETU were 6.6 µg/L and 4.3 µg/L respectively. This study has not yet been published but the results were provided by Lindh (Mail communication, November 2008). In another unpublished study regarding exposure of the general population in south of Sweden mean and median of urinary ETU levels were 0.5 µg/L and 0.8 µg/L respectively (Lindh, Mail communication, November 2008).

There are many sources for exposure of EBDCs and ETU in the general public. According to Aprea et al. (1996) ETU can for example be found in cigarettes and wine. Residues of the Mancozeb or other EBDCs in foodstuffs may be transformed into ETU during cooking and industrial processing (Watts et al., 1974). Other authors claim that certain processing procedures such as peeling and heating can help reduce pesticide residue levels (Hwang et al., 2002). An experimental study performed by Knio et al. (2002) examined degradation of EBDCs to ETU and the persistence of ETU on tomato. ETU content was reduced by about 80 % by day 20 after the fungicide application. Knio et al. (2002) also demonstrated a 70 % decrease of ETU when washing the tomatoes in water. Further losses of ETU occurred during boiling (6%) and during storage of the tomato paste for a period of 3 weeks (3%).

3.5 Toxicity of Mancozeb and ETU

Both Mancozeb and ETU have shown relatively low acute toxicity in experimental studies with values at LD_{50} > 5000 mg/kg for Mancozeb and 2000 mg/kg for ETU (U.S. EPA, 2005 pp. 13, 15). However,

there are strong indications for various adverse health effects concerning Mancozeb and ETU, both derived from experimental studies as well as epidemiological studies. Many of the risk concerns for Mancozeb and the other EBDCs are driven by risk from ETU (U.S. EPA, 2005 p. 30). The studies presented below concern both ETU and Mancozeb.

3.5.1 Experimental studies

Experimental studies on ETU and Mancozeb are listed in the Appendix B. The most prominent effects from experimental studies will be presented in this chapter.

3.5.1.1 Experimental studies on Mancozeb

U.S. EPA (2005 p. 11) demonstrates that the thyroid gland is the target organ for Mancozeb and thyroid toxicity has been manifested as alterations in thyroid hormones, increased thyroid weight, and microscopic thyroid lesions, and thyroid tumors. The carcinogenicity of Mancozeb was examined more closely by Belpoggi et al. (2002) where Mancozeb caused an increase in total malignant tumors. There are also studies that show evidence of a correlation between skin cancers amongst mice as a result from dermal exposure to Mancozeb (Mehrotra et al., 1987; Shukla et al., 1990). Furthermore in two Indian studies on rats it was shown that high intake of Mancozeb affected the estrous cycle (Mahadevaswami et al., 1999; Baligar & Kaliwar, 2001). Cecconi et al. (2007) suggest with regard to both in vitro studies on mouse embryos and a reproductive in vivo study on mice (Rossi et al., 2006), that Mancozeb has an effect on female infertility and that it also can induce ovarian cancer.

In vitro studies on rat brain cells exposed to Mancozeb, have shown various neurological effects (Vaccari et al., 1999; Leiphon & Picklo, 2006). These effects could, according to the authors, be associated with development of parkinsonlike symptoms. Vaccari et al., (1999) accentuates that even if the effect dose was high in their experiments, prolonged exposure to lower doses might lead to neurological effects. Debbarh et al. (2002) argues that experimental studies have shown an increased neurotoxicity of the herbicide Paraquat in combination with Mancozeb.

3.5.1.2 Experimental studies on ETU

ETU which is considered to be more toxic than Mancozeb have induced a wide spectrum of anomalies in many test animals (Houeto et al., 1995). In one of the studies that U.S. EPA (1996) base their toxicity profile for ETU on, which was performed by Graham et al. (1975), thyroid carcinogenicity was evident. The carcinogenicity of ETU was also manifested in a study by Chhabra et al. (1992) and the thyroid gland in rats and mice and the liver in mice were identified as target organs of ETU toxicity.

According to FAO/WHO (www.inchem.org refer to Teramoto 1978) ETU is considered to be heavily teratogenic in rats while non teratogenic in mice. In a study on rats performed by Lu and Staple (1978) where the dams were administrated ETU, a majority of the fetuses had malformations. In a teratogenic study on cats no malformations where found on the fetuses but the dams had adverse effects on the central nervous system (Khera & Iversen, 1978). Neurotoxic effects have also been studied in rats, where the peripheral nervous system was affected (Arbetslivsinstitutet, 2001 p. 8 refers to Ugazio et al. 1985).

Furthermore, ETU has been the subject for many in vitro and in vivo studies for genotoxicity (FAO/WHO, www.inchem.org). FAO/WHO (www.inchem.org) concluded that ETU is a non genotoxic

agent. According to Dearfield (1994) who collected and evaluated different short time studies on genetic effects up to 1993 considers ETU to be a weak genotoxic agent.

3.5.2 Epidemiological studies and other observations in humans

Evidence for effects in humans is less well founded (Lindh et al., 2008). Since ETU is excreted through the urine within hours and not accumulated, is it difficult to make any conclusions about the link between past time exposure and effects (Nieuwenhuijsen, 2003 p.13). Concerning occupational exposure to pesticides it can also be difficult to differentiate what substance that leads to a specific effect since workers normally are exposed to a vast quantity of different compounds in different combinations (Leiphon & Picklo, 2006). Little is known about additive, protective or synergic effects of combinations of pesticides. However, some important results can be seen, mainly concerning effects on the thyroid gland.

A few epidemiological studies have shown a correlation between occupational exposure of Mancozeb and effects on the thyroid glands. The study on banana plantation workers in the Philippines indicated that high exposure to Mancozeb correlated with enlarged thyroid glands (Panganiban et al., 2004). Steenland et al. (1997) found an increase in thyroid-stimulating hormone among Mexican workers exposed to EBDCs which can have adverse effects on the thyroid glands. They also found increases in sister chromatoid exchanges as well as in frequency of chromosome aberrations, which also is demonstrated in another study conducted by Jablonická et al. (1989). An increased level of chromosome aberrations has been shown to be predictive for cancer in humans (Hagmar et al., 1994). In a large study on Norwegian farmers no association between Mancozeb exposure and thyroid cancer were found to exist (Nordby et al., 2005). However, the same study found a moderate relationship between Mancozeb exposure and neural tube defects.

Effects in exposed children

Falk-Filipsson et al. (2007) stress that the timing of the exposure may be just as important as the dose in determining the potential toxicity of a chemical. They emphasize that some organ systems, for example the endocrine, reproductive, immune and nervous systems, show specific vulnerability to chemical toxicity during development as organ maturation is an ongoing process until adulthood. A suggestion is that an attempt should be made to perform fetus- and child-specific risk assessments for chemical substances to which they are likely to be exposed.

Neurological effects of Mancozeb exposure, which have been shown in test animals, have also been subject for examination regarding human exposure. Leiphon and Picklo (2006) and Debbarh et al. (2002) argue that epidemiological studies indicate a correlation between Parkinson's disease with living in a rural area and/or exposure to agricultural pesticides. Vaccari et al. (1998) suggest that prolonged exposure to Mancozeb might lead to neurological symptoms. In an epidemiological study performed by Kimura et al. (2005), workers exposed to Mancozeb showed a slightly higher incidence of various neurologic effects than non-exposed workers. In the previous study in the banana village, an effect associated with ETU was oppositional disruptive behaviour disorders in boys (van Wendel de Joode et al., 2008).

Moreover, acute effects that have been observed are that Mancozeb can cause irritation on the skin, in the respiratory tract and in the eyes (U.S. EPA, 1999). The substance has also been shown to be responsible for some cases of chronic skin disease amongst exposed workers (U.S. EPA, 1999).

3.5.3 Carcinogenicity of Mancozeb and ETU

U.S. EPA (2005 pp. 18-19) has classified Mancozeb and ETU as a group B2 carcinogen which means that it is *possibly carcinogenic to humans*. IARC downgraded ETU from group 2B to group 3 (*not classifiable as carcinogenicity to humans*) in 2001 (www.iarc.fr). They concluded that there is inadequate evidence for the carcinogenicity of ETU in humans, but there is sufficient evidence in experimental studies for the carcinogenicity in animals. This decision was criticized by Steenland (2003) who emphasizes that the one epidemiological study performed at that time, which had looked at altering homeostasis in thyroid glands found evidence for carcinogenicity in humans.

3.5.4 Conclusions of the Toxicity of Mancozeb and ETU

Houeto et al. (1995) summarizes epidemiological and experimental studies on EBDCs and ETU and draw conclusions about the toxicity of Mancozeb and its metabolite to humans. They argue that the different toxic responses seen among various animal species are best explained by differences in their metabolism of EBDCs. This implies that one cannot easily extrapolate findings from animals studies directly to humans and since few studies of exposure to EBDCs have been conducted in humans, the current knowledge of the subject must be considered quite limited. Nevertheless, they conclude that these chemicals exert at least some toxic effect in humans, especially certain classes of workers for whom the exposure is chronic, and there is reason to at least suspect possible carcinogenicity of these agents in humans.

4 Risk theory

The ISO/IEC (2002) defines risk as the combination of the probability and the consequences of an event. According to the definition of risk by Kaplan and Garrick (1981) the risk can be analyzed by answering three questions: what can happen, how likely is it and what are the consequences?

4.1 Risk assessment

To answer these questions in a structured way a risk assessment process can be applied. According to ISO/IEC (2002) the risk assessment is a process where the risk is analyzed and evaluated (*Figure 9*). With the starting point in Kaplan and Garrick's questions a three step process can be outlined for the assessors: define what can happen, identify scenarios when it can happen and measure the severity and impact of that happening (Kolluru et al., 1996 p. 2.3). By using this three step process the risk analysis as well as the risk evaluation from ISO/IEC (2002) would be accomplished.

Risk assessment

- **Risk analysis** where the risk is identified and estimated according to its probability and consequence
- **Risk evaluation** where the risk is compared to certain risk criteria to decide the signification of the risk.

Figure 9. *Risk assessment according to ISO/IEC (2002).*

Comparing the ISO/IEC (2002) process with the frameworks for environmental and health risk assessments, the latter consist of three parts instead of two: *problem formulation, risk analysis* and *risk characterization* (U.S. EPA, 1992a; NRC, 1983; WHO, 2001). The risk analysis consists of a dose-response assessment and an exposure assessment. The risk evaluation phase from ISO/IEC (2002), where the risk is compared to a certain risk criteria, is included in the risk treatment.

Risk assessment

- **Problem formulation**
- **Risk analysis**
  - Dose response assessment
  - Exposure assessment
- **Risk Characterization**

Figure 10. *Risk assessment according to U.S. EPA (1992a), NRC (1983) and WHO (2001)*

4.1.1 Vocabulary

U.S. EPA, WHO and NRC all have different vocabulary for the different parts in the risk assessment. For example U.S. EPA refers to exposure characterization instead of exposure assessment which is the expression used in this thesis. In this thesis the different parts of the risk assessment are named as described in Figure 10, independently from which organization or agency a particular method or statement is taken from.

4.2 Risk management

The process to define the risks *and* coordinate activities in order to reduce them is called risk management (ISO/IEC, 2002). Risk management, in contrast to the scientific process of risk assessment, involves making decisions concerning actions in response to estimated risks to humans

or ecological systems (WHO, 2001 p20). The risk management process adds two parts to the previously presented risk assessment (*Figure 11)*.

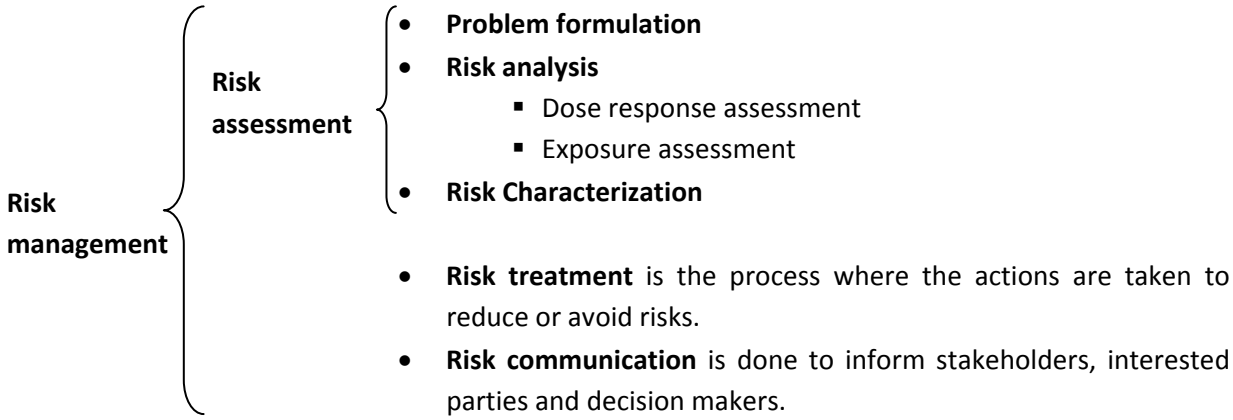

Figure 11. *The risk management process inspired by ISO/IEC (2002), NRC (1983), WHO (2001) and U.S. EPA (1992a).*

Aspects of health risk assessment methods are more closely presented in Chapter 5 since that is the focus of this thesis. Below is a short description of the risk treatment and the risk communication. These two parts will be discussed in relation to this study in Chapter 8.

4.2.1 Risk treatment

The risk treatment is the procedure to choose methods and implement actions to modify the risk and it implies either acceptance or reduction of it (ISO/IEC, 2002). Risk evaluation, is according to ISO/IEC (2002) a part of the risk assessment. However, in this thesis, the aim of the risk assessment is to present an unbiased risk estimation. Thus, the risk evaluation will be a part of the risk treatment discussion in this thesis. There are several different criteria for risk evaluation, i.e. whether or not a risk is acceptable and if it should be reduced. For further reading about risk criteria, see Mattson (2000, pp. 69-70) who mentions criteria based on rights and criteria based on cost-benefit calculations which are the most known criteria. Risk reduction can, according to ISO/IEC (2002), be achieved by reducing the consequence, reducing the frequency or both.

4.2.2 Risk communication

Risk communication is by NRC (1989 p. 2) described as an interactive process of exchange of information and opinions among individuals, groups and institutions. These exchanges typically involve the transfer of information about risks from experts to non-experts (NRC 1989 pp. 2-3). The content of this risk information generally takes the form of, facts or hypotheses about the level of risk that exists within a system; the meaning of the risk relative to other issues of concern or decisions and actions or policies that may be undertaken to manage or control it (Joseph, 2007 refers to NRC, 1983). Risk may be perceived very differently between different individuals depending on the person's relation to the risk, culture, earlier experiences, etcetera (Sjöberg, 2000). Hence it is of great significance that the information is communicated with that in mind. Forget and Lebel (2001) emphasizes that when studying communities, with a view to risk reduction, it is not enough to know the sources and effects of pesticide exposure. Knowledge of the cultural and social context allows researchers to understand the behavior of local actors and to get insight in pathways of exposure (Forget & Lebel, 2001). NRC (1983 p. 3) claims that many people, including some scientists, decision makers, and members of the public, have unrealistic expectations about what can be accomplished

by risk communication. According to them, risk management decisions that benefit some people harm others. In addition, people do not all share common interests and values, so better understanding may not lead to consensus about controversial issues. But even though good risk communication cannot always be expected to improve a situation, poor risk communication will nearly always make it worse (NRC, 1983 p. 3).

5 Risk assessment methods

This thesis will focus on the risk assessment and hence a closer introduction to different assessment methods will be introduced in this chapter.

As mentioned above, the risk assessment framework used in this thesis has three major parts (*Figure 12*). The risk analysis consists of two parts that are equally important to the analysis and the risk assessment as a whole (NRC, 1990 p. 35).

Risk assessment

- **Problem formulation** Goals for the study are set, methodic is chosen and restrictions identified
- **Risk analysis**
  - **Dose-response assessment** The effects from an exposure is characterized
  - **Exposure assessment** The exposure is characterized
- **Risk characterization** The exposure and the dose-response assessment are combined to estimate the overall risk

Figure 12. *Framework for the risk assessment used in this thesis*.

5.1 Problem formulation

The problem formulation is the first phase of the risk assessment where goals and focuses are set for the project (U.S. EPA, 1992a p. 9; WHO, 2001 p. 8). The outcome from the problem formulation should be a model that identifies the endpoints of the study, data needed, methods to be used and restrictions of the assessment (U.S. EPA, 1992a p. 9). An endpoint can be anything that the risk assessment aims to protect, for example the health of the children (U.S. EPA, 1992a p. 2). However since many endpoints are not directly measurable, measurement endpoints must be identified which are related to the endpoints of the assessment (U.S. EPA, 1992a p. 9). An example of this could be *health* as an assessment endpoint and *days of illnesses* as measurement endpoint.

Probabilistic analysis

Probability can result from variability and uncertainty (Suter, 2007 p. 4). In risk assessments, probability is often semi quantified and handled with safety factors or with conservative alternatives that are chosen (Suter, 2007, p. 4). Many of the estimations that must be made when making a decision have an uncertainty and yet assumptions are often treated as exact numbers (Burgman, 2005 p. 181).

By using a probabilistic approach to the risk assessment the variability and uncertainties are taken into account by using distributions instead of point estimates (Hart et al., 2003).

5.2 Risk analysis

The risk analysis consists of two parts, dose-response assessment and exposure assessment (NRC, 1983). The dose-response assessment evaluates the relationship between different doses/exposures and the magnitude in the response (U.S. EPA, 1992a, p.22). The exposure assessment estimates the exposure by measurements or by models (WHO, 2001 p.11). The two assessments must be performed interactively to assure that effects found in the dose-response assessment are compatible with the biota and exposure routes identified in the exposure assessment (U.S. EPA, 1992a p. 17).

This implies that extrapolations often must be made in both the dose-response assessment as well as in the exposure assessment (U.S. EPA, 1992a pp. 12, 22).

5.2.1 The dose-response assessment

In this section the principles of and methods for dose-response assessment are presented. The dose-response assessment investigates the effects of the chemical and extrapolations are made from data to fit for the purpose of the assessment. Often the purpose of the assessment is to estimate a Rfd (Reference dose), a daily dose to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of harmful health effects during a lifetime (U.S. EPA, 1996 www.epa.gov). Hence, there will be a focus on the theory and methods behind assessing a Rfd. Data for the assessment can be found by looking at results from epidemiological studies or from experimental studies on animals (Skerfving, 2001 p. 11). Both kinds of studies are associated with difficulties and many uncertainties when extrapolating from them.

5.2.1.1 Data collection

Data from human epidemiological studies have the advantage over toxicological studies in animals that no extrapolation from animals to humans has to be made in the risk assessment (Falk-Filipsson et al., 2007). However as Skerfving (2001 p. 11) emphasizes exposure is often uncertain which consequently makes it difficult to include them in a quantitative assessment.

According to Falk-Filipsson et al. (2007) toxicological studies with experimental animals are the main basis for risk assessment of chemicals. For most toxicants except genotoxic carcinogens, it is generally assumed that there is a threshold dose below which no toxic effects will occur (Cecconi et al., 2007). In animal experiments, the threshold may be approximated by the no observed adverse effect level (NOAEL) (Falk-Filipsson et al., (2007).

5.2.1.2 Uncertainties regarding the dose-response assessment

Uncertainties when extrapolating results from experimental studies can originate from several different sources. For example experimental studies may be inadequate and substantial differences between the potency of chemicals may be masked by a small sample size and large experimental variations (Burgman, 2005 pp. 173-174). Also, often only a few different species are used in experimental studies so the diversity is not very wide (Burgman, 2005 p.186). Falk-Filipsson et al. (2007) summarize aspects that increase the demand of assessing uncertainties in dose-response assessment when extrapolating from experimental data:

- quality of the toxicological database
- the nature of the effect
- when lacking a NOAEL, extrapolating from LOAEL to NOAEL
- route-to-route extrapolation
- the duration of the exposure
- inter-species differences in sensitivity
- intra-species differences in sensitivity

There is a need to evaluate the ***quality of the data*** that is used in the dose-response assessment, for example; number of doses used in the experiment, what interval between them and if the result is significant or not (Falk-Filipsson et al., 2007; Bengtsson, Personal communication, October 2008).

Transparent expert judgment of the quality of the database in a case-by-case manner is important when considering the database.

A broad definition of an ***adverse effect*** is: ‘‘changes in morphology, physiology, growth, development or lifespan of an organism which result in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences’’ (WHO/IPCS b, www.inchem.org). Some effects can be considered as more severe than others according to Falk-Filipsson et al. (2007). EU, for example, considers carcinogenicity, mutagenicity and reproductive toxicity as more severe than other endpoints.

NOAEL is by definition one of the experimental doses tested and not necessarily the same as the ‘‘true’’ no adverse effect level (NAEL) (Falk-Filipsson et al., 2007). Falk-Filipsson et al. (2007) question the use of LOAEL/ NOAEL ratios to estimate NAEL from LOAEL, since doses in toxicological tests are usually spaced at fixed intervals. Moreover, Burgman (2005 p. 178) argue that the doses tested in experimental studies are often higher than found in the environment and it is hard to foresee the effects from high to low doses. The dose-response curve usually follows a curve that is linear or logarithmic, but there exists also chemicals with healthy effects at low levels and adverse effects at higher levels (Burgman, 2005 p. 178).

Most toxicity studies are performed using oral exposure which is also the main ***route of exposure*** in humans for pesticide residues or contaminants in food and drinking water (Falk-Filipsson et al., (2007). However, for many chemicals, and most occupational exposures, the predominant routes of exposure in humans are through the skin or inhalation (Falk-Filipsson et al., 2007).

Falk-Filipsson et al. (2007) state that the most relevant experimental ***duration of exposure*** to base a risk assessment upon, is one that imitates the human exposure situation as well as possible. In many cases a lifelong exposure is the most relevant exposure scenario for humans, also for strictly conservative reasons (Falk-Filipsson et al., 2007). Hence, extrapolations should be done upon result from lifetime animal studies (in practice a chronic study) (Falk-Filipsson et al., 2007). However, for numerous substances data is only available from acute (<2 weeks), subacute (2–4 weeks) or subchronic (1–3 months) experimental studies (Falk-Filipsson et al., 2007).

Even with adequate experimental studies the difficulty to extrapolate the results to ***other species*** than the ones the experiment is conducted upon still remains. Although qualitative and quantitative differences exist between various mammalian species, there are fundamental similarities which justify the assumption of comparability of biological processes (Falk-Filipsson et al., 2007). However as Falk-Filipsson et al. (2007) hold, it should be noted that there are also species specific differences in for example metabolic patterns that make extrapolation between species difficult.

Within a species the sensibility to a chemical may vary a lot as well, for example; pesticides may have substantially different health consequences for children depending on the developmental stage during which the exposure occurs (Fenske et al., 2005). Human susceptibility to chemicals may depend on a number of determinants, including for example the genotype and gender of the individual, age, growth and development, health status and lifestyle factors (Falk-Filipsson et al., 2007). Particular attention should be paid to effects on the nervous, reproductive, endocrine and immune systems and also the metabolic pathways, all of which in part develop new functional properties during childhood (Falk-Filipsson et al., 2007).

5.2.1.3 Dealing with the uncertainties

It is obvious that when assessing a Rfd there are various steps that imply difficult judgments regarding uncertainty in the assessment. Suter (2007, p. 359) accentuates the urge for methods when extrapolating from few data to units and responses that constitute the assessment endpoints. According to Suter (2007, p. 359) the most commonly used methods are assumptions, factors and statistical models. Some of the methods described in the literature are presented in this section.

Assessment/safety/uncertainty factors are often used to compensate for uncertainties that originate from extrapolations from the NOAEL in animals to humans and to compensate for inter-individual differences in human sensitivity (Falk-Filipsson et al., 2007; WHO, 2001 p. 16). Many different factors have been proposed for different purposes (Falk-Filipsson et al., 2007) but often a tenfold factor is used for every extrapolation made (Suter, 2007 p. 361). Falk-Filipsson et al. (2007) review different factors proposed in the literature and suggest their best choice for every extrapolation. Many argue that these factors are based on assumptions and have no scientific value (Suter, 2007, pp. 361). Suter (2007, pp. 361) holds that the primary advantage of using factors is their ease, while their primary disadvantages are that they are largely subjectively derived and the result is often considered safe even though not clearly associated with a certain effect.

An alternative approach is to consider each assessment factor as uncertain and characterized as a random variable with a log-normal ***distribution*** with a mean and a standard deviation (Std) (Falk-Filipsson et al., 2007). By using a probabilistic approach to the risk assessment the variability and uncertainties are taken into account by using distributions instead of point estimates (Hart et al., 2003).

Another way of assessing dose-response relationships is presented by Suter (2007, pp. 362-364) and is called ***species sensitivity distribution (SSD).*** This method was developed for ecological risk assessment in order to protect some proportion of species. A percentile of the distribution of the test endpoints values for various species can be used to represent a dose that would affect a percentage of that community. Aggregation of species within a common distribution provides more data with which to define the model. They may be interpreted as distributions of the probability that a species will be affected at a particular dose and also as an estimate of the distribution of sensitivities of species within a community.

Further on, Suter (2007, pp. 366-367) presents a method that according to him is rarely used, but extremely flexible and quantitatively rigorous; ***regression models***. Regressions of one species on another, one life stage on another, one test duration on another can be used to extrapolate between various tests endpoints and assessment endpoints.

There are also other more sophisticated statistical methods for dose-response assessments, for further reading see Suter (2007, chap. 26).

5.2.1.4 Reference doses of agencies and organizations for ETU

When U.S. EPA calculated a Rfd for ETU they used assessment factors to extrapolate the results from an animal study to be valid for humans, see Appendix C. They recommend a Rfd of $2 * 10^{-4}$ mg/kg/day.

FAO/WHO (1993 www.inchem.org) used assessment factors as well and recommends a reference dose of $2*10^{-3}$ mg/kg/day. IPCS (1993 www.who.int/ipcs) recommends an Rfd of $4*10^{-3}$mg/kg/day.

How this dose has been extrapolated has not been found in literature. For all reference doses see table 2.

Table 2. *Reference data for ETU*

Organization	Reference dose	Safety factors	Study
U.S. EPA (2005)	$2*10^{-4}$ mg/kg/day	Af= 1000	Dog study
FAO/WHO (1993)	$2*10^{-3}$mg/kg/day	AF=100	Dog study
IPCS (1993)	$4*10^{-3}$ mg/kg/day	unknown	unknown

5.2.1.5 Assessing cancer potency factor for ETU

Carcinogenicity of a substance can either be assessed with a non-linear threshold approach or a linear non-threshold approach depending on the mechanism of the tumor formation (U.S. EPA, 2005 p. 18). With a non-threshold approach the toxicant is given a potency factor $(mg/kg/day)^{-1}$ instead of a Rfd (Timbrell, 2002, pp.175-176). The potency factor is multiplied with a daily dose (mg/kg/day) in order to calculate the risk, which must not exceed 10^{-6} (U.S. EPA, 2005 p. 21). U.S. EPA (2005, pp. 18-19) uses a non-threshold linear approach for assessing the cancer risk. The same cancer potency factor is used for both Mancozeb and ETU since Mancozeb's carcinogenicity is considered to originate from the metabolite ETU (U.S. EPA, 2005 p. 21). The cancer potency factor given is 0.0601 $(mg/kg/day)^{-1}$ (U.S. EPA, 2005 p. 19).

5.2.2 Exposure assessment

In the exposure assessment the levels of the chemical is measured or modeled and the actual exposure of organisms through contact and uptake is estimated (Suter, 2007 p. 197). There are several different approaches to how the exposure can be approximated. Nieuwenhuijsen (2003 p. 12) divides them into two groups, indirect methods and direct methods.

Indirect methods

- Environmental monitoring
- Questionnaires and interviews
- Records and data
- Diaries

Direct methods

- Biological monitoring
- Personal monitoring

Environmental monitoring is done by measuring the levels of the chemical in different media which the target is exposed to (Jönsson, 2001 p 18). This kind of approach miss to take into account all uptake routes and only the exposure where the measurements are performed will be assessed (Jönsson, 2001 p 18).

Questionnaires and interviews can be used when the exposure cannot be measured or at very large studies (Nieuwenhuijsen, 2003 p. 21). In many epidemiological studies it is necessary to examine past exposure to correlate with present effects, for example cancer (Espinosa et al., 2005; Nieuwenhuijsen, 2003 p. 16) and hence no direct methods or environmental monitoring are applicable.

Medical ***records*** or ***data*** on the amount of sold or imported chemicals in a region are other methods to estimate past exposure (Monge, 2006 pp. 17-18).

With ***biological monitoring*** the internal levels of a chemical or its metabolites in the body are examined. The concentration of a contaminant is most commonly measured in blood and urine (NRC, 1991 p 119). The advantage with this method is that all uptake routes in all environments will be included (Hoet & Haufroid, 1997; NRC, 1991 p. 116). However it is not possible to find out where and how the exposure occurs with only this method (NRC, 1991 p. 118). To overcome this, additional environmental measurements, questionnaires or an activity pattern diary can be carried out to receive more information about the exposure routes (Lioy, 1995).

Personal monitoring is a method where personal exposure is measured rather than the exposure in the environment (Nieuwenhuijsen, 2003 p.23). This can be done by attaching a monitor to the person instead of measuring the media where they live or work.

Exposure to children in farm environments

In farm environments, people are exposed to pesticides through several pathways: inhalation from drift or re-suspension of contaminated soils, skin exposure from contaminated surfaces and ingestion exposure through hand to mouth contact (Curwin, 2006).

Children living in agricultural communities or children, whose parents work with pesticides, may be exposed through additional pathways (Fenske et al., 2005). Differences in the behavior of children, for example that they are closer to the ground could enhance exposure to pesticides (Cohen Hubal et al., 2000). Differences in exposure between children and adults are partly due to higher metabolism and larger surface to volume ratio in children (Cohen Hubal et al., 2000). They differ from adults by having a higher potential for exposure due to small body mass and different intake, less developed metabolism and higher rates of cell production and growth (Landrigan, 2004).

5.2.2.1 Uncertainties regarding the exposure assessment

It is important for every exposure assessment to identify and describe all the uncertainties that are associated to that assessment (U.S. EPA, 1992b p. 3). Uncertainties regarding the exposure assessment are in many ways similar to the ones emphasized in the dose-response assessment. The ***data*** collected in the exposure assessment may not be comprehensive, eventual ***extrapolations*** that are necessary in order to make the exposure assessment compatible to the dose-response assessment are associated with uncertainties. Often, many of these uncertainties cannot be quantified but can only be subject for a qualitative discussion.

5.3 Risk Characterization

The risk characterization is the last step of the risk assessment. This is where the risk is estimated by combining the results from the dose-response and the exposure assessment and the uncertainties from the analysis are presented (U.S. EPA, 1992a p. 28, NRC, 1983).

WHO (2001 p. 17) describes an approach for the risk characterization which is divided into three steps. First the results from the assessments of exposure and dose-response are combined to estimate the risk. Secondly the uncertainties related to the two assessments are discussed and thirdly the results are summarized and presented in a form that is easily understood by stakeholders and risk managers, without losing their accuracy.

5.3.1 Integration of dose-response and exposure assessment

Three general approaches are presented by U.S. EPA (1992a p. 22) to conduct the first step, the integration of the dose-response and the exposure assessment. These three are: comparing a single potency value to the exposure values, comparing distributions of effects and exposures, conducting a simulation model.

A single potency value is often produced by the use of safety factors and is divided by the exposure to get a quotient (U.S. EPA, 1992a p. 28). If the quotient is 1 or higher an adverse effect is likely to occur. This approach is according to U.S. EPA (1992a p. 28) the least probabilistic of the three approaches. A more probabilistic approach is the one where distributions of the exposure and the effects are compared (U.S. EPA, 1992a p. 29). The greater overlap between the two distributions the greater the risk. The third and last approach; conducting a simulation model is still a relatively new approach and is mostly used for population modeling.

5.3.2 Uncertainty analysis

The uncertainty analysis, which is the second step in the risk characterization, identifies, and when it is possible, quantifies the uncertainty in the problem formulation, risk analysis and the risk estimate (U.S. EPA, 1992a p. 30). The analysis of uncertainties should result in an evaluation of the impact of the uncertainties on the overall risk assessment. Uncertainties that should be considered could originate from natural variability, incompleteness of data, errors and finally that a conceptual error exist in the problem formulation and hence the whole assessment has the wrong focus (U.S. EPA, 1992a pp. 30-31).

5.3.3 Result presentation

The last step of the risk assessment is the presentation of the results. Suter (2007. p. 433) presents two methods where the first one divides the risks into those that need immediately attention and those that can be ignored for the moment. The second is called definitive assessment and provides risk estimates for all considered risks. According to U.S. EPA (1998 p. 99) the completion of the risk characterization is where the risk assessors should clarify the relationships between stressors, effects, and ecological entities and reach conclusions regarding the occurrence of exposure and the adversity of existing or anticipated effects. This could really only be done with the last of the two methods suggested by Suter (2007 p. 433). The result can be two cumulative curves, one that shows the variability of effects and the other which shows the variability in exposure (U.S. EPA, 1998 p. 108).

6 Methods used in this thesis

In this chapter, methods used for the risk assessment in this thesis are presented. Uncertainties derived from the risk assessment are discussed in Chapter 7.3.

6.1 Problem formulation

The problem formulation was an iterative process and the objectives, methodic and restrictions were constantly developed during the course of time for this thesis. The goals and restrictions were presented in the task description and the methodic is described in this chapter. The endpoint studied in this thesis was the health of the children living in this banana village and the measurement endpoint was the urinary ETU levels.

6.2 Dose-response assessment

The purpose of the dose-response assessment is to estimate a Rfd for ETU (highest acceptable daily intake) and present existing ones. General methods when performing a dose-response assessment have been presented in the chapter 5.2.1. In addition to that, the method used by U.S. EPA (2005 p. 17) in estimating a Rfd for ETU is presented in Appendix C. However, described methods use safety factors to estimate a safe dose which has been criticized for being non scientific and subjectively derived (Suter, 2007, p. 361; Falk-Filipsson, 2007). Thus this chapter will present a combination of the methods presented previously in order to estimate a Rfd for ETU in a more probabilistic way. This estimation will be comparable to that of U.S. EPA. The aim is to construct a transparent model and to document all the uncertainties that are represented in it, as well as the ones that are not.

Later in the risk characterization all the Rfds of U.S. EPA, WHO and IPCS and the one estimated in this chapter will be used. No estimations for a potency factor for the carcinogenic approach will be done, but the factor U.S. EPA suggests is used in the risk characterization.

6.2.1 Model

The general model was a construction of a ***SSD (species sensitivity distribution)*** for mammals' sensitivity to ETU. The approach was that the dose for chronic exposure at which 99 % of all mammal species are protected is considered to be a safe dose for humans without accounting for intra-species variations. An approach of that kind requires a large amount of data from chronic studies. Since experimental data regarding exposure to ETU were found for only five different mammal species, other species somehow had to be introduced into the model. ***Regression models*** were constructed in order to estimate toxicity to ETU for other mammal species. This was done by searching U.S. EPA's database (*chemical toxicity information for aquatic and terrestrial life,* www.epa.gov/ecotox) for other substances that have been used in experiments with a greater number of mammal species. The toxicity of these substances was then compared with the toxicity of ETU by comparing specific endpoints for ETU with the same endpoints for the other substances. The ETU data base includes many experiments on rats and mice, thus endpoints such as LD50, LOAEL and NOAEL for specific effects for those two species were used. Primarily data bases for the EBDCs and other fungicides were evaluated and secondly other well known, vastly used pesticides.

Because these chemicals were tested for other species and there was a correlation between the toxicity of ETU and these chemicals more species could be introduced in the SSD. The species that

were possible to introduce into the model are presented in table 3. In the table, the five animals already tested for ETU are presented as well.

Table 3 *shows the different species introduced in the model and what extrapolations that had to be done to transform the dose into a chronic NOAEL of exposure to ETU.*

Species	Substance	LOAEL/NOAEL	Time duration
Mouse	ETU	NOAEL	Chronic
Rat	ETU	NOAEL	Chronic
Dog	ETU	NOAEL	Chronic
Cat	ETU	LOAEL	Subacute
Hamster	ETU	NOAEL	Subacute
Sheep	Chlorpyrifos	LOAEL	Subchronic
Rabbit	Chlorpyrifos	NOAEL	Subchronic
Goat	Malathion	NOAEL	Acute
Cattle	Malathion	LOAEL	Acute

The aim was to construct a SSD with these species, where each species represented a dose that corresponds to a chronic NOAEL of exposure to ETU. In order to estimate these doses, regression models were constructed between different pesticides and between NOAEL and LOAEL. To account for the uncertainties in the regressions, distributions of the regressions were applied (*Figure 13*).

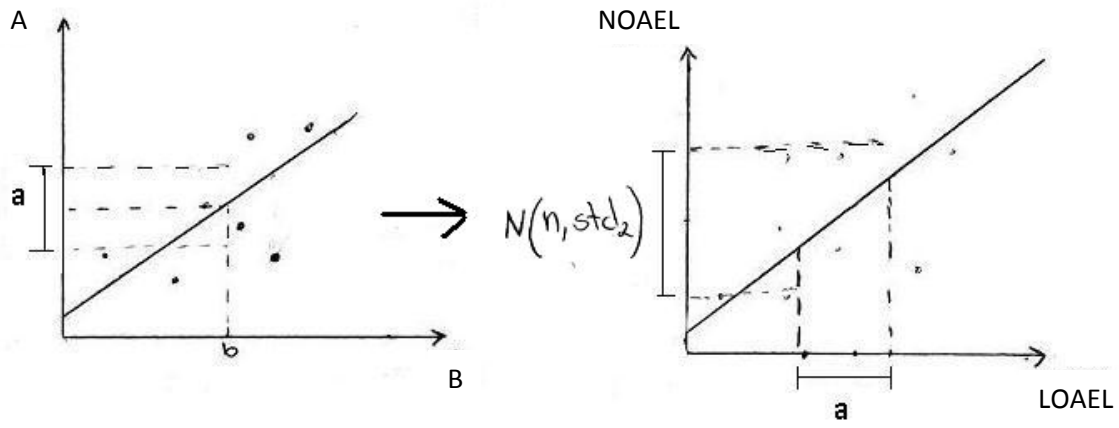

Figure 13. *The first extrapolation could be from chemical B to chemical A. The mean and the standard deviation of that regression (a) is placed in the other regression in order to get a NOAEL for chemical A, in this case ETU.*

Moreover assumptions were made and ***probabilistic assessment factors*** were used to compensate for the difference in time duration. Uncertainty analysis concerning the assumptions and extrapolations were carried out and is presented in Chapter 7.3.1.2.

6.2.2 Regression models

Regression models were constructed between different pesticides and between NOAEL and LOAEL. A record over all experiments used in the regressions is found in Appendix E.

6.2.2.1 From other substances to ETU

Correlations between ETU and other well known pesticides were examined. Primarily correlations were searched for between ETU and the EBDCs but there was insufficient data for these and due to that correlations with other pesticides were examined. Among pesticides tested on many species there were weak correlations between ETU/Malathion and ETU/Chlorpyrifos. It was not possible to search for correlations between effect doses for other animals than mouse and rat because there were not enough studies performed for other animals. There was also a high variety in exposure type, such as dermal, oral, injection etcetera, between different studies and due to that, many experiments were not comparable. Concerning time duration difference, only experiments performed within the same class of time were compared in this study (chronic, subchronic, subacute, acute). Some studies expressed the doses in ppm instead of mg/kg. In those cases a conversion factor of 15 % for mice and 7.4 % for rats were chosen (www.epa.gov; Decos, 1999). The linear regressions between the two correlations are presented in figure 14 a and b.

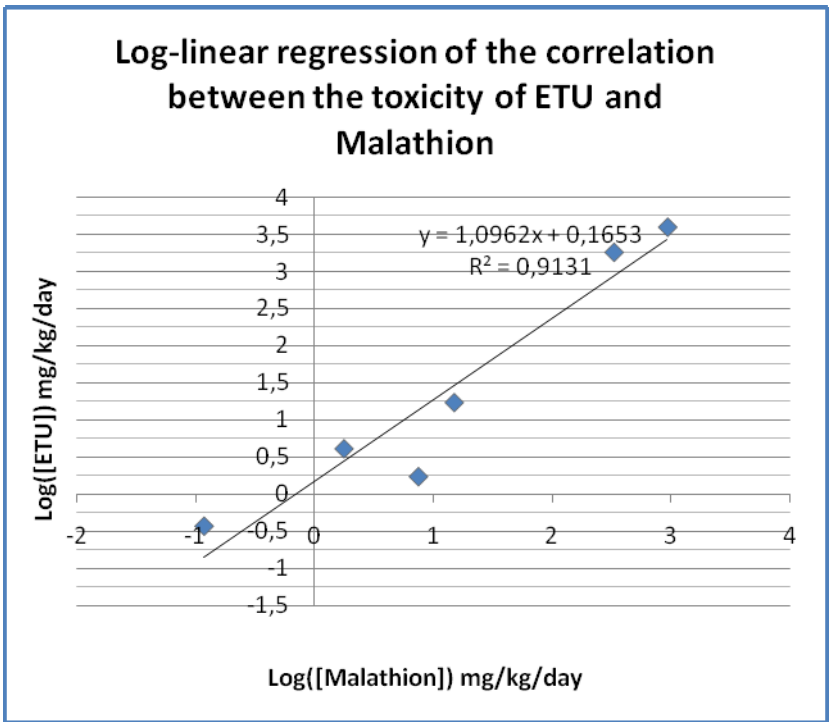

Figure 14 a. *The figure shows the log-linear correlation between the toxicity of ETU and Malathion in mice and rats.*

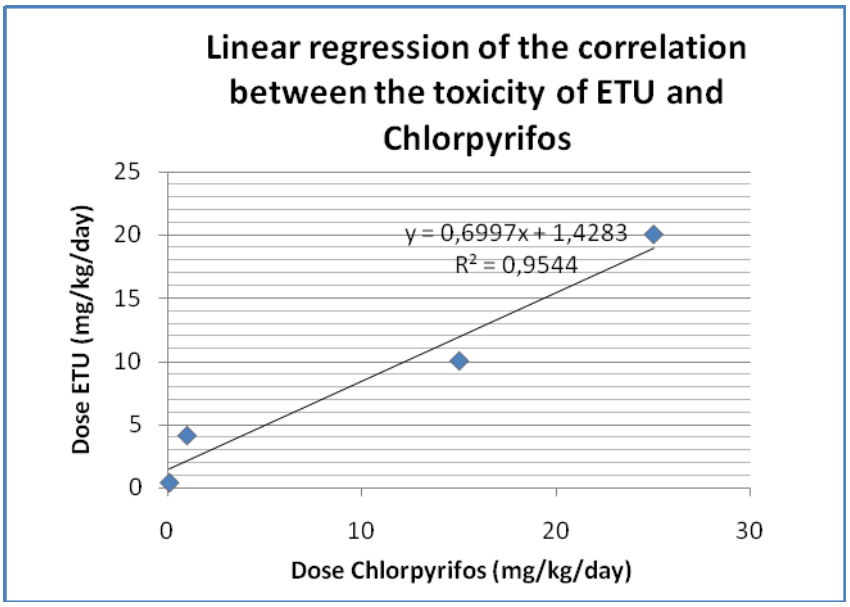

Figure 14 b. *The figure shows the linear correlation between the toxicity of ETU and Chlorpyrifos in mice and rats.*

If the comparison included a LD50 value, it had a great impact on the gradient and a log-linear model was chosen to reduce the impact of the LD50 value on the regression (this was used for ETU/Malathion regression). Since the test animals used for the Chlorpyrifos/ETU regression showed a good relationship at low levels of exposure, the LD50 value was neglected and there was no need to use logarithmic values. A normal distributed standard deviation was calculated for the regression in order to account for uncertainties in the relationship.

The correlations now made it possible to transform the results from studies conducted with Malathion and Chlorpyrifos to ETU values and thus to include four new species into the model. These species were presented in table 3. However in order to derive a Rfd some further extrapolations had to be made since the model was for chronic exposure and NOAEL.

6.2.2.2 LOAEL to NOAEL

A similar approach was therefore used to assess a NOAEL when only a LOAEL was given in the study. Comparisons were made between NOAEL and LOAEL values derived from the same studies containing at least four doses. The data was collected from studies on five different pesticides, seven different species and various differences in time duration. As in the case with the ETU/Malathion regression a few high values determined the linear regression; however the log-linear regression (*Figure 15*) better described the relationship between LOAEL and NOAEL in the whole interval. A normal distributed standard deviation was calculated for the regression in order to account for uncertainties in the relationship.

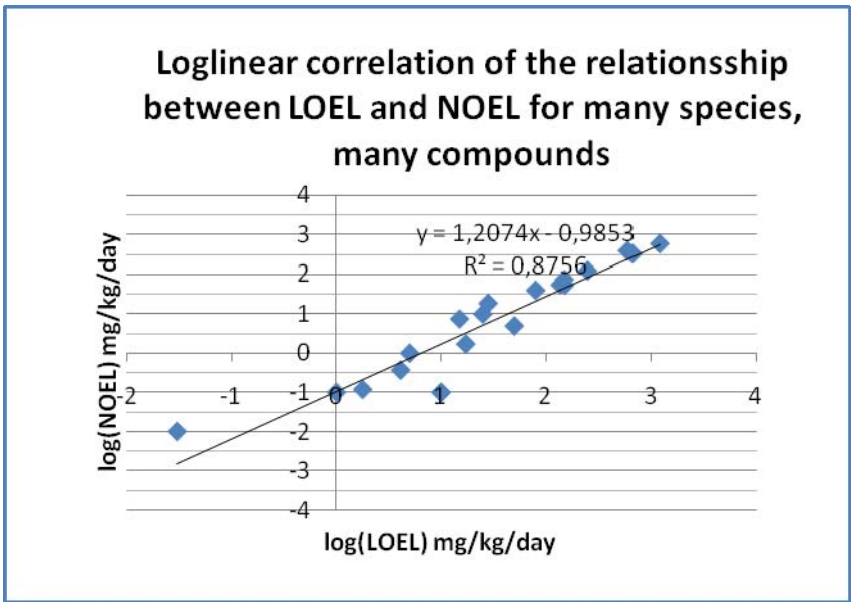

Figure 15. *The figure shows the Log-linear correlation between the LOAEL and NOAEL for many species and many compounds.*

6.2.3 Time duration differences

The third factor of substantial importance was the time duration of the studies where chronic NOAELs were to prefer. Therefore when the studies were shorter, extrapolation factors had to be used in order make them comparable to chronic studies. Thus and in accordance to Falk-Filipsson et al. (2007) a factor 7 was used to cover for 95 % of differences in NOAEL between subchronic and chronic values. When extrapolation between subacute and chronic study, a factor 39 was used to cover for 95 % of the differences in NOAEL, also this assumption is in accordance with Falk-Filipsson (2007). Both these approaches were based on probabilistic described assessment factors, but were only using a conservative value of it and not the whole interval. There is no guidance in the literature in how to assess extrapolation factors for acute to chronic studies, but by testing different values on the factor it was concluded that the result was not very sensitive to variations. Thus a uniform distribution was applied which spanned between safety factors from 100 to 1000.

6.2.4 Constructing the SSD

The extrapolations described above made it possible to calculate a dose for four new species, now expressed as chronic NOAEL from exposure to ETU and together with the original values for ETU the SSD could be constructed. The NOAELs corresponding to mouse, hamster, rat and dog were estimated as point values since their experiments resulted in NOAELs which were conducted for ETU. However, the extrapolated values were included in the calculations as distributions. Calculations are presented in Appendix E. A Monte Carlo simulation was performed for each species. The distributions had to be truncated in order to not result in any negative values. A distribution was then constructed with the compiled results and the 1st percentile was estimated from the distribution (*Figure 16*).

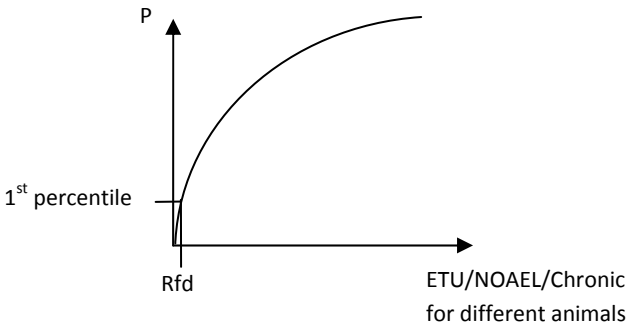

Figure 16. *The constructed SSD showing the 1st percentile.*

6.2.5 Intra-species variations

Finally to account for intra-species variations an additive inter-individual assessment factor was used to extrapolate to the rfd. In accordance with Falk-Filipsson et al. (2007) a factor 15 was applied to compensate for differences in metabolic pathways between individuals and other sensitive groups such as pregnant women and elderly people. Moreover, Falk-Filipsson et al. (2007) suggest an extra safety factor between 1-10 when performing child-specific risk assessments depending on toxicological properties of the chemical and the quality of the database. In this assessment, the child-specific assessment factor was estimated to 10 due to conservative reasons. Hence in total, an additive factor 15 * 10 = 150 was applied to the 1st percentile value from the SSD described above.

Figure 17 show the complete model used in this thesis for estimating the Rfdused. The resulting Rfd is presented in Chapter 7.1.

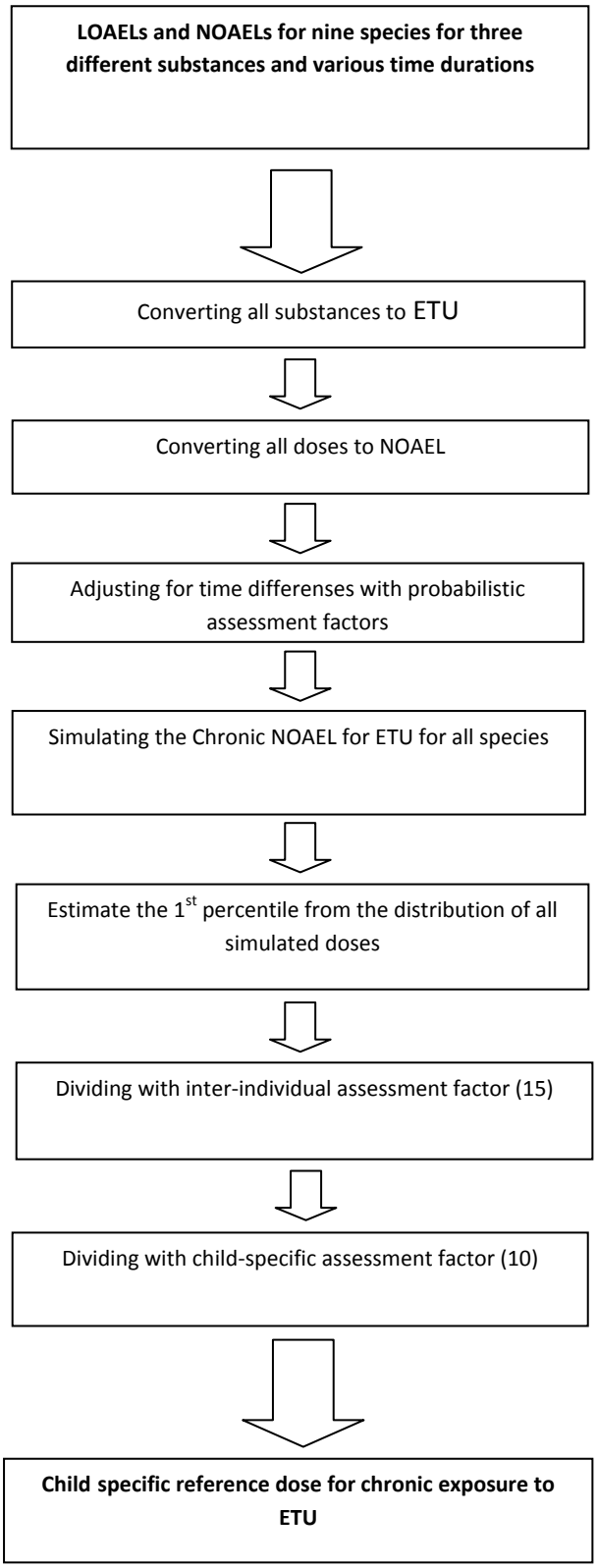

Figure 17. *An overview of the complete dose-response model used in the thesis.*

6.3 Exposure assessment

The exposure assessment in this study was based on biological monitoring. However, as stated in Chapter 5.2.2, in order to know where the exposure originates from supplementary observations must be performed, which was done in the following way.

Field study

- Biological internal monitoring - urine sampling
- Observations on where the spraying occurred
- Questionnaires

Analytical method

- Analysis of the urine for ETU

Exposure analysis

- The ETU levels in the urine was translated into an oral dose
- Distributions were fitted to the data of the exposure
- Statistical analysis of factors inducing high exposure

According to Fenske et al. (2005) biologic monitoring appears to be the best available method for assessment of children's exposure to pesticides. Even though the interpretation of urinary metabolites can be difficult it is probably the best approach to capture exposure variability of non persistent pesticides in young children, partly because of the ease of the collection (Fenske et al., 2005). Fenske et al. (2005) stress though the need of repeated measurements, to overcome high intra-individual variability of biologic samples for most pesticides in use today. In this study this was overcome by repeated measurements for seven consecutive days.

6.3.1 Field study

The field study was performed during three visits in the village. During the first and second visit there was no collection of urine samples. The purpose of those visits was to prepare for the main study by getting to know the people in the village, observe how the spraying occurred and work out strategies for how the main study would work practically.

6.3.1.1 Urine sampling

The study group consisted of 37 children, 19 boys and 18 girls, at the ages 8-10 years old. All these children were included in the former study on pesticide exposure (Van Wendel de Joode et al., 2008). The present study also included the children's parents of which 55 contributed. All parents signed an inform of consent concerning both them and their children. The aim and performance of the study were explained to all involved before the start of it.

A sample of the first morning urine was collected from each child during a period of seven consecutive days. A total of 21 spot samples were collected during day 2 and 3 between 9 a.m. and 2 p.m. Urine samples were also collected from the parents of the children during the first four days of the study period. A schedule over the parents and children and how much they participated, both in the urine collecting and for questionnaires, are presented in tables in Appendix D.

The school day was divided into two parts where half of the class had morning classes and the other half had afternoon classes. Children brought their samples to school when they started. If a child had forgotten to bring the sample he or she was asked to immediately hand in a new sample. A notation was made that the sample was collected later. As for the parents, no attempts were made to receive a new sample due to practical difficulties and respect of their privacy. The children who had afternoon classes were asked to keep their and their parents' samples in the refrigerator. Notations were made when there were suspicions that the samples were not the first morning urine. The suspicions could be based on warmth or a very small amount of urine or a resistance to go and get the sample in their home.

The samples were brought by the children in small plastic containers and the urine samples were poured into a test tube. The test tubes with the urine were first cooled down in a cooling box in field and frozen in the evening of the collection. This was considered to be a safe method by Lindh (Mail communication, July 2008).

The children received a new bag with cups and containers for the next day's samples when they handed over the urine samples of the day. The bag also included a small toy that worked as a motivator. On the last day of the field work all children received small prizes. The urine containers had to be cleaned and reused once since there were not enough containers for all samples. They were cleaned once with normal tap water and twice with distilled water to rinse out all urine. This method was approved by Ruepert (Personal communication, July 2008) and Lindh (Mail communication, July 2008).

All collected samples were sent off for analysis of ETU content at the laboratory of the Division of Occupational and Environmental Medicine, Lund University, where methods have been developed for analyzing and detecting metabolites at low levels (Lindh et al., 2008).

6.3.1.2 Observations

To be able to correlate the urinary ETU levels to different factors, several hypotheses were concluded prior to the main field study. The hypotheses dealt with different factors that were thought to influence the exposure. These factors are illustrated in figure 18 and the main hypotheses can be found below.

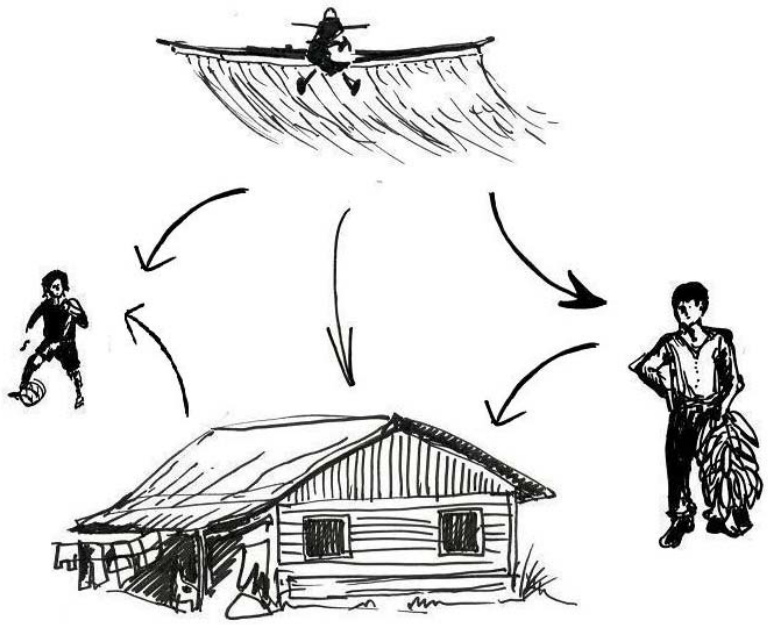

Figure 18. *The different ways of exposure to Mancozeb and ETU that were considered in the exposure assessment.*

Following hypotheses were concluded before the main field study:

- The ETU levels in the children will be significantly higher after a spraying day than after a non spraying day.
- There will be a significant difference between ETU levels in the children who have been thought to be exposed and the children who have not.
- There will be a significant correlation between the level of ETU in the children and how close they live to the banana plantations. (Leonél Córdoba Gamboa assisted with the distances)
- There will be a significant correlation between the level of ETU in the parents and their children.
- There will be a significant difference in ETU levels depending on the task performed in the banana company. This difference will be seen in both the children and their working parents.

To be able to test the hypotheses, observations were made while in field which were used when assessing factors that induce high ETU levels. The following observations were made during the main study:

- The location and duration of the spraying was marked on a map
- White papers were placed on the school yard to verify drift from nearby spraying activities
- Questionnaires were given to the parents about:
  - What company the parents work at
  - Their duties in the company
  - Whether or not they brought home clothes from work

The questionnaires also contained a question about how long the families had lived in the village to get an idea of for how long the children had been exposed.

Checklists were developed during the first visit and during the pilot study in order to perform the observations efficiently.

6.3.1.3 Analytical method

The method for analyzing the ETU in the urine was developed by Lindh et al. (2008) and is using liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS). The LOD (limit of detection) for ETU was set to a 0.05 ng/ml. All samples were analyzed in duplicates, and the mean value was calculated. The levels were corrected for creatinine content and density. This method is considered by Lindh et al. (2008) to be appropriate for analysis of ETU as a biomarker. For more information about the analytical method the article *Analysis of ethylenethiourea as a biomarker in human urine using liquid chromatography/triple quadrupole mass spectrometry* by Lindh et al. (2008) is recommended.

In order to account for the urinary dilution the levels of ETU can be adjusted for either creatinine or density. For all samples the density and the creatinine levels were determined. However, according to Lindh et al. (2008) when comparing populations or individuals with large differences in muscle mass, various ages and both females and males are included, density adjustment is the more applicable. Hence, in this assessment the levels adjusted for density were used.

6.3.1.4 Estimating the oral dose of ETU

In order to make the exposure assessment compatible with the dose-response assessment and be able to compare the urinary ETU levels to the Rfds, the levels had to be converted to corresponding oral doses. This extrapolation was done by the help of a urinary level/dose correlation from a human experimental oral exposure study by Lindh et al. (2008). In the experiment two healthy volunteers received 8.9 µg/kg b.w. of a commercial fungicide containing 64 % Mancozeb and 4.5 % ETU in a single oral dose. This corresponds to a consumption of 5.7 µg/kg b.w. of Mancozeb and 0.4 µg/kg b.w. ETU. All their urine voids were collected during 104 post-exposure hours and examined for ETU. The peak urinary level was 4 hours after the exposure and the elimination half-life at 19 hours for the female and 23 hours for the male, density adjusted.

Three different approaches were chosen to convert the urinary ETU levels to oral doses with the help from the correlation from the study by Lindh et al. (2008):

Approach 1
Collection was made 4 hours after the exposure

Approach 2
Collection was made 12 hours after the exposure

Approach 3
Collection was made 24 hours after the exposure

These approaches were established on the hypothesis that the excretion rate in children follows the same pattern as the two test persons in the study by Lindh et al. (2008), i.e. the urinary levels decrease over time from the exposure moment. Approach 1 was based on the assumption that the collection was made at the urinary peak level, i.e. 4 hours after the oral exposure (Lindh et al., 2008). Since the collection was made every 24 hours, approach 3 was based on the assumption that oral exposure occurred just after the collection of the day before, and is considered to illustrate the highest possible dose. With these three approaches the most and the least conservative alternatives were taken into account as well as an alternative in between. The children's exposure is probably very different from the exposure in the study by Lindh et al. (2008), both regarding exposure route

and that there was only one moment of exposure in that study. Hence the three approaches used in this thesis cannot be perceived as the reality but rather as a method in order to include uncertainties and variability.

All ETU levels were converted to oral doses in accordance with all three approaches contributing to three different sets of data. To estimate the percentage of the children in the village who are at risk the data sets were compared to the Rfds and the cancer potency factor was used to calculate the cancer risk.

6.3.1.5 Estimating what causes the exposure

In order to evaluate what causes high levels in the children, a statistical analysis was carried out with regard to the observations from the field study and the answers from the questionnaires. The results will be presented both graphically and with statistical measurements, with the help of Excel and SPSS.

To see if there are any correlations between observations, measurements or other variables the Spearman's test can be used , which is a non-parametric test and hence useful when the distribution of the data is unknown (Wahlgren, 2005 p. 95). With this test the values are ranked and the correlation is calculated between the ranking numbers. The correlation factor can be between -1 to 1 depending on if the correlation is positive or negative and the significance of the correlation factor is also given.

Another non-parametric test that can be useful is the Mann-Whitney U test (Wahlgren, 2005 pp. 116-117). This is a test to see if there is a significant difference between two groups. The result of this test will only give the significance and not the ranking of the two groups. It is thus important to investigate further which group has the higher rank. If there are more than two groups a Kruskal-Wallis test can be used (Wahlgren, 2005 pp 118-119). This test will rank the different groups and tell if there is a significant difference between any two of the groups. However, to be sure between which two groups there is a difference, the Mann-Whitney U test can be used.

Graphical methods were used to look for correlations and to visualize differences between groups. Also the mean, standard deviation, maximum, minimum and median were calculated for the ETU levels depending on day, exposure grade and other grouping variables.

Correlations were searched for between:

- Children and their fathers
- Children and their mothers
- Mothers and Fathers
- Children and the distance from their house to the banana plantations
- Mothers and the distance from their house to the banana plantations
- Children and their given exposure grade

Significant differences were searched for between:

- Individuals among the children
- Different days for children
- Fathers working in the field and fathers not working in the field
- Children with fathers with different occupation

- Mothers with husbands with different occupation
- Mothers with different occupation
- Boys and girls

6.4 Risk characterization

In the risk characterization the calculated oral doses from the exposure assessment were compared with the Rfds from the dose-response assessment to see how big percentage of the children who exceeded the Rfds, and also the percentage of children who exceeded the highest acceptable cancer risk at 10^{-6} was estimated Furthermore, the uncertainties which were derived from the risk assessment were discussed and evaluated. In this assessment the integration of the dose-response and the exposure assessments will be presented after the uncertainty analysis.

6.4.1 Uncertainties in the risk assessment

The uncertainties from the integration were discussed together with all other uncertainties derived from the risk assessment.

6.4.2 Presentation of the integration of the dose-response and exposure assessments

The single potency values from the dose-response assessment (the Rfds) were compared to the three distributions of oral doses, approach 1, 2 and 3. To find out the cancer risk the cancer potency value was multiplied with the oral doses. The results were compared to the highest acceptable risk; 10^{-6}.

The factors that significantly induced high exposure were presented.

The presentation of the results should be done by a definitive approach. This implies presenting all the results and giving the risk managers an unbiased risk estimation.

7 Results from the risk assessment

In this chapter, results from the dose-response and the exposure assessment are presented as well as the integration of them in the risk characterization. There will be a discussion of the results from the statistical analysis and also a discussion about uncertainties in the risk assessment.

7.1 Results from the dose-response assessment

The dose-response assessment that was performed for ETU for nine different animals and their NOAEL for chronic exposure resulted in a distribution that can be seen in figure 19.

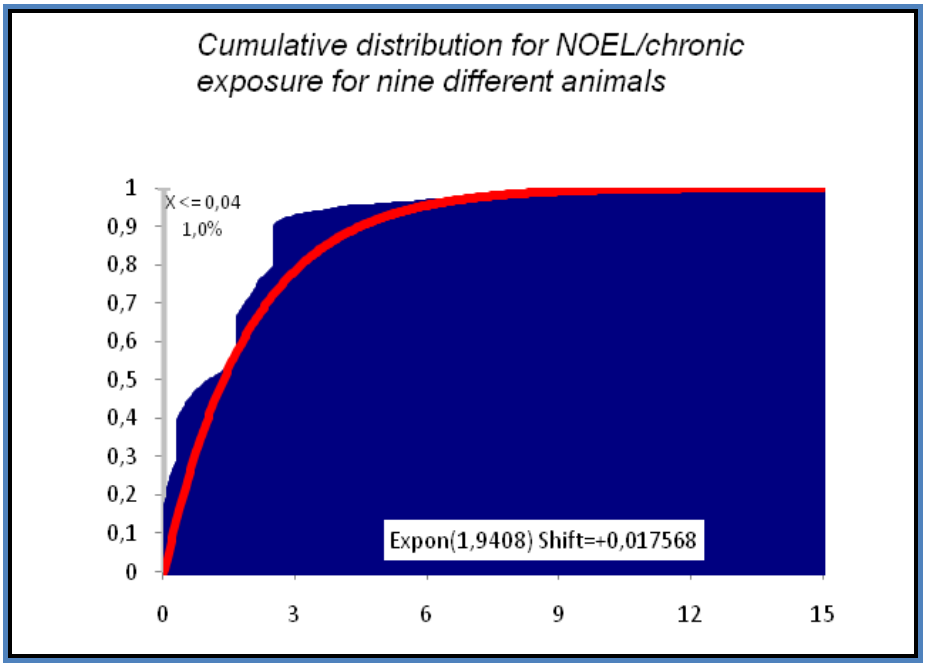

Figure 19. *The result from the dose-response assessment.*

In table 4 Rfds from U.S. EPA, FAO/WHO and IPCS are presented together with the estimated value from this thesis, i.e. the 1st percentile of the distribution. The input value was chosen over the value from the distribution function for further extrapolations, which is supported by Suter (2007 p. 364). This value was further divided with assessment factors for intra species differences (assessment factor 15) and for children specifically (assessment factor 10).

Table 4. *The different Rfds that will be used in the risk characterization*

Origin	Rfd
U.S. EPA (2005)	0.0002
FAO/WHO (1993)	0.002
IPCS (1993)	0.004
From this thesis	
Distribution, 1st percentile	0.04
Input, 1st percentile	0.018
Intra species adjusted (AF 15)	0.0012
Children (AF 10)	0.00012

7.2 The results from the exposure assessment

Results from all parts of the exposure assessment are presented in this chapter.

7.2.1 From field study

During the main field study that lasted all together 8 days, spraying observations were performed day 0-6 and urine samples collected day 1-7.

7.2.1.1 Urine samples and questionnaires

An overview of all the urine samples collected during the field study is presented in Appendix D, where the questionnaires are included as well. In table 5 a summary over the number of collected urine samples are presented. In total 37 children, 26 mothers and 21 fathers agreed to leave their urine. Altogether, including the 21 spot samples, 433 samples were collected.

Table 5. *The number of urine samples that were received every day, excluding the spot samples.*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Children	35	36	37	37	37	35	37
Mothers	20	23	23	22	-	-	-
Fathers	14	19	20	18	-	-	-
Total	69	78	80	77	37	35	37

7.2.1.2 Spraying observations

In Table 6 below, a summary of spraying activities, weather and important observations during the week is presented.

Table 6. *Observations on sprayings made during the field week. Urine samples were collected on day 1 to day 7.*

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Morning-spraying	Far away on Chiquita side, northwest	-	Short while in the far west, Del Monte side	-	Close, both sides in the northwest part	Close, both sides in the southeast part	Close, south of East part of village
Other observations	-	-	-	-	Many children on their way to school, when spraying occurred	Many still sleeping on the Del Monte side	On a Sunday, drift in to the village
Afternoon-spraying	Very close to school, Del Monte side, probably drift	Very Far away on Chiquita side	-	-	In the middle of the village, Del Monte side	Del Monte side , north west part of the village	-
Other observations	Many children in School	-	-	-	Children playing outside	Children playing outside	-
Weather	Sunny	Rainy in the morning	Rainy in the afternoon	Heavy Rains	Clear/ Cloudy	Clear/ Cloudy	Sunny

Valuable observations include:

- There were spraying activities on a Sunday, which was not expected.
- At some occasions, spraying started before the plantation begun.
- Windy conditions could make the pesticides drift in to the village.
- At day 7, when the last urine samples were collected, there were heavy spraying activities over the school again. Unfortunately, no urine samples were collected the day after. However an attempt in assessing exposure to Mancozeb/ETU by placing white paper on the ground at six spots around the school showed that there was substantial drift from the spraying. The papers were clearly dotted with yellow substance. Considering the amount of substance on some papers, there might even have been direct spraying over the parts of the school closest to the banana plantation.
- Many children did not wear shoes when playing outside.
- According to the answers from the questionnaires at the Chiquita side, workers are allowed in to the plantation 1 day after spraying, while on the Del Monte side, only 1 hour is required.
- The fathers working directly with pesticides were provided working clothes from the companies, while the others working in the field had their own clothes.

7.2.2 Results from the chemical analysis

The following results from the chemical analysis are preliminary data and not the final data, since there was not enough time to wait for those.

The ETU levels in the children, fathers and mothers for the whole week are presented in Table 7. As can be seen in the table, median values are consistently lower than the means. This implies that there is not an even distribution of the levels, but the majority have rather low levels, while some levels are substantially higher which affect the mean. It can also be seen that fathers have the highest ETU levels, while mothers have the lowest.

Table 7. *Descriptive statistics for ETU levels in fathers, mothers and children.*

Descriptive Statistics for ETU levels						
Type	**N**	**Minimum**	**Maximum**	**Median**	**Mean**	**Std. Dev.**
Children's levels	274	1.15	19.41	6.05	6.60	3.44
Fathers' levels	71	1.46	41.53	13.34	14.67	11.24
Mothers' levels	88	0.68	14.88	4.03	4.76	2.87

To better illustrate the actual exposure in the village, a distribution over the ETU levels in the children was constructed (*Figure 20*). In this distribution, all children's samples from all seven days were included. As can be seen, and which also was indicated in the mean/median comparison, the majority had low levels while some had higher.

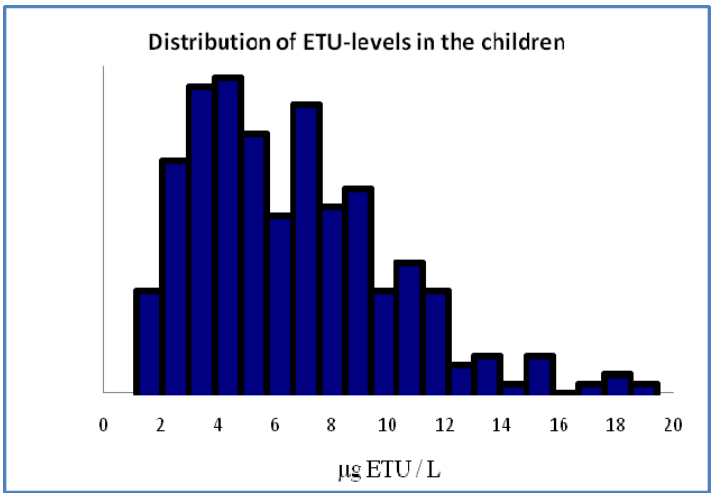

Figure 20.*The distribution of ETU levels in the children for all seven days.*

The individual differences for the children will be subject for a discussion later in this chapter.

7.2.3 Calculated oral doses

The approach described in the method chapter generated three possible doses for every child, where the first represent the least conservative assumption, and the last represent the most conservative assumption. Distributions of doses of ETU for the children for the three different estimations on duration from exposure are displayed in Figure 21.

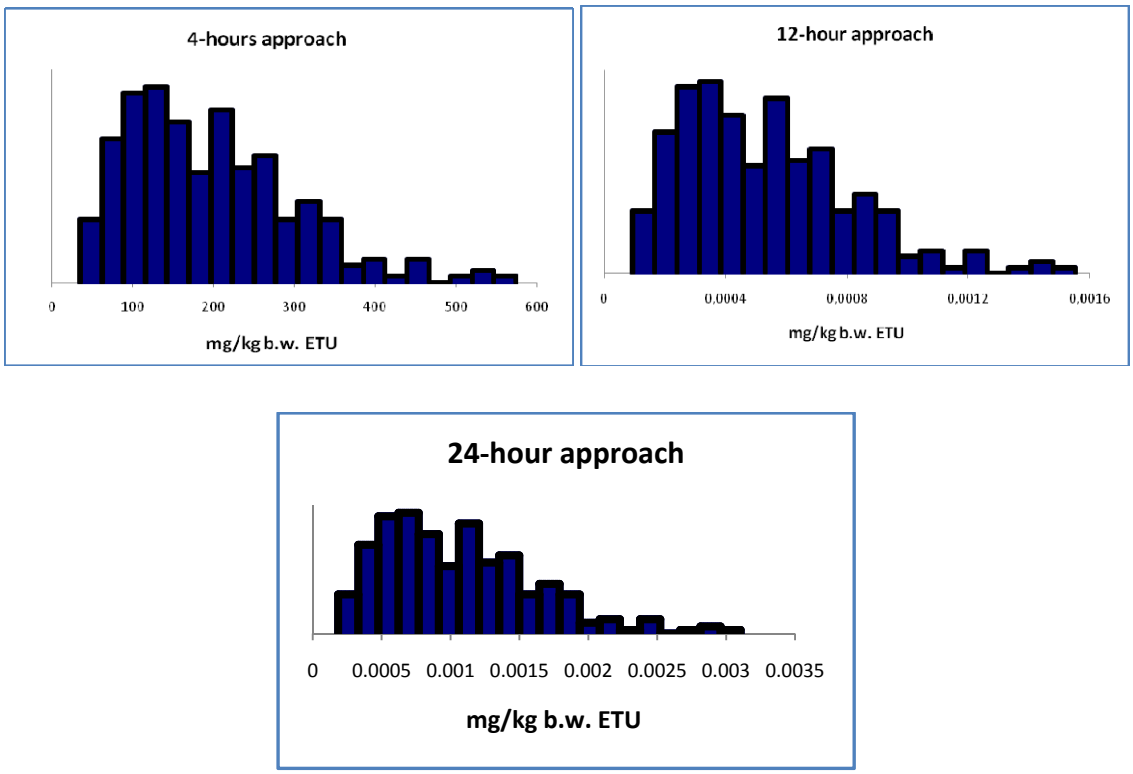

Figure 21. *The distribution of the approximated doses if using the three different approaches*

7.2.4 Results from statistical analysis

In this section the results from the statistical analysis are presented. The levels of ETU in the children are correlated with possible exposure factors that have been presented earlier in this thesis. In some cases, exposure factors regarding the parents are investigated. This is done in order to better understand the exposure situation for the children and will not be a subject for discussion concerning parental exposure itself.

7.2.4.1 Spot samples

Firstly in order to better understand the exposure for the children the difference in ETU level between the spot sample and the morning sample of the same day and child was evaluated. The spot samples that were collected day 2 and day 3 between 9 a.m. and 2 p.m. were compared with corresponding first morning urine samples. The difference in ETU level (morning sample - spot sample) between the compared samples are displayed in Figures 22.

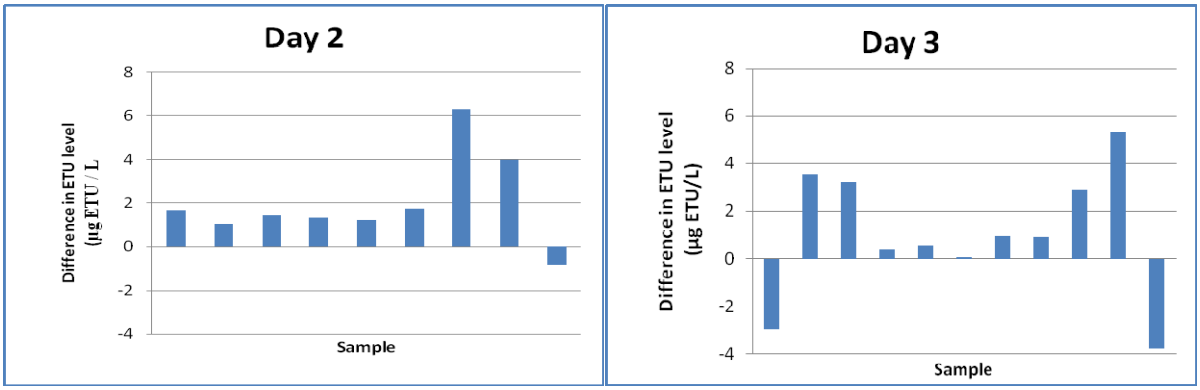

Figure 22. *Difference in ETU-level between the morning sample and corresponding spot sample day 2*

When the difference between the samples is positive, this would reflect an uptake during the day before and when the difference is negative it would reflect an uptake closer in time. The fact that some of the differences are negative indicates that the children could have a more or less continuous exposure. The spot samples were only included when calculating the overall risk for the children, but excluded in the further statistical analysis.

7.2.4.2 Difference in ETU levels depending on days

The ETU levels in all children were compared with day of collecting as grouping variable. Means of the ETU levels for the different days are presented in Figure 23. As seen in the Figure, highest means were found day 1 and 2, whereas day 4 was characterized with the lowest mean. Descriptive statistics for the different days are displayed in Table 8 together with spraying data.

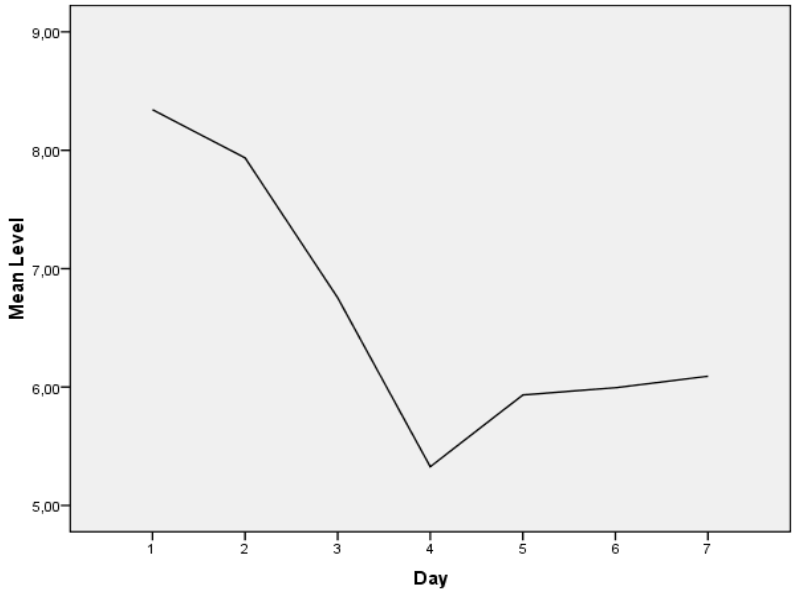

Figure 23. *The mean of the ETU levels in all children the different days.*

Table 8. *Descriptive statistics for ETU levels with day as grouping variable*

Descriptive statistics for ETU levels in the children the different days								
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Mean	-	8.35	7.93	6.75	5.33	5.96	5.98	6.09
Std. Dev.	-	4.32	3.68	2.88	2.45	2.75	3.59	3.70
Median	-	7.30	8,10	6.38	5.30	6.08	5.43	5.26
Minimum	-	2.04	2.15	2.95	1.83	1.33	1.15	1.44
Maximum	-	17.70	12.76	11.51	13.20	17.39	18.27	19.41
Spraying	close	far away	far away	none	Close	close	close	-

It was suspected that individual differences in for example metabolic functions could mask the result so the individual differences were examined. In Figure 24, the individual ETU levels during the seven days are shown. It is obvious that there are substantial individual differences between the children. Some children vary a lot between the days, while some have similar levels all seven days.

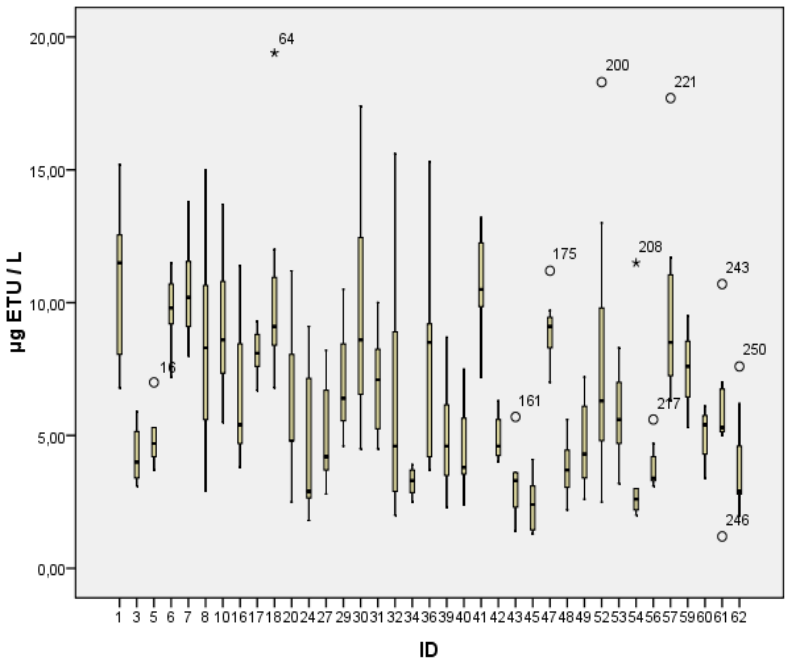

Figure 24. *All Individual ETU-levels for the seven days. The midline is the median, the box represent 50 % of the values, the span represent all the values, the rings represent outliers, while the asterisks represent extreme values*.

Figure 25 demonstrate the levels of ETU different days. Here, as well, it is obvious that within one day levels vary a lot between the children.

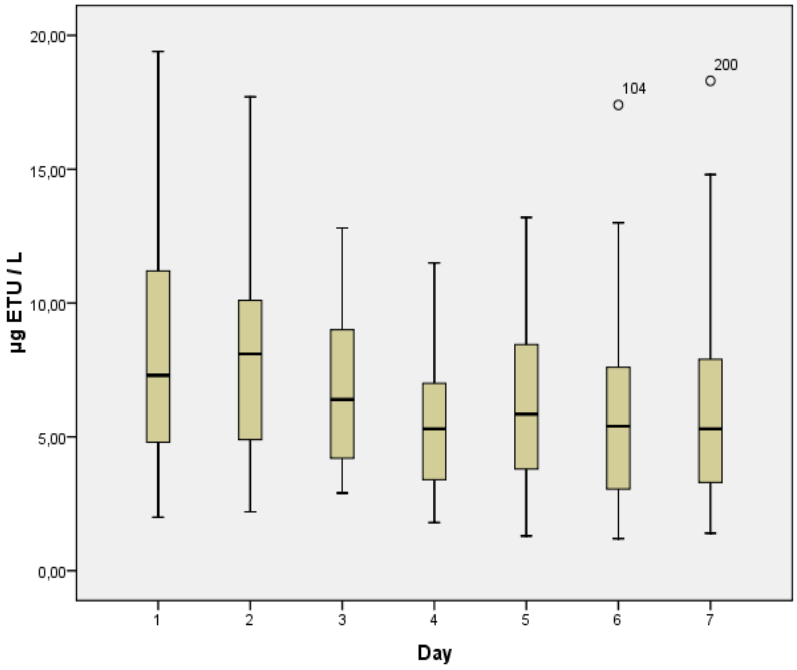

Figure 25. *All ETU levels with day as grouping factor. The midline is the median, the box represent 50 % of the values, the span represent all the values and the rings represent outliers.*

In order to mitigate this potential problem, the ETU-levels were ranked between the days within the individuals. The lowest level one individual had during the seven days was ranked as 1 and the highest as 7. This way, individuals with consistently higher levels would not have an impact on the result. The mean of the ranking numbers for the different days are displayed in Figure 26. This

approach shows a similar result as the previous one and it can be concluded that levels are the highest day 1 and 2 and the lowest day 4. Although in this case the levels are higher on day 5 than on day 6, while it is the opposite when just comparing the levels instead of their rank.

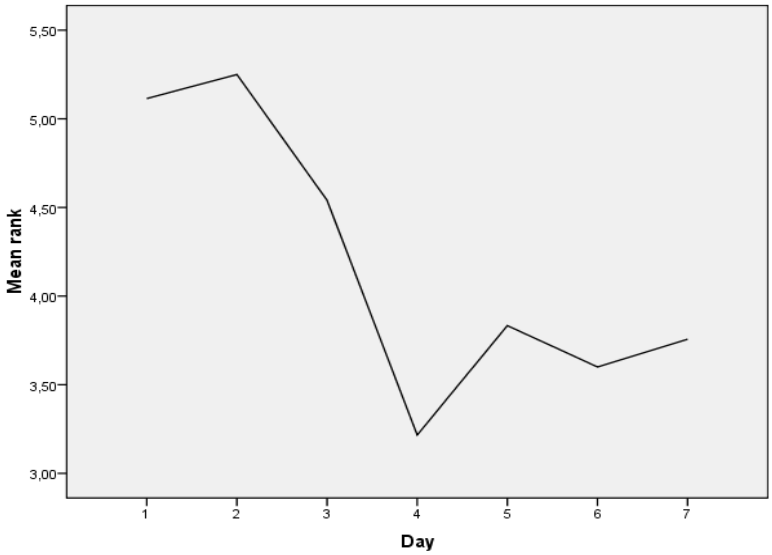

Figure 26. *The means of the ranked levels day 1 - day 7.*

The differences in ETU levels between the different days were tested with Mann-Whitney U. The results from the test are presented in Table 9, and significant differences are represented with asterisks.

Table 9. *The results from a non-parametric test, Mann-Whitey U on the difference in ETU level between the days in all children (* = p<5%, ** = p<1%).*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 1	-	0.836	0.169	0.002**	0.022*	0.018*	0.015*
Day 2	0.836	-	0.206	0.001**	0.023*	0.017*	0.017*
Day 3	0.169	0.206	-	0.036*	0.247	0.143	0.170
Day 4	0.002**	0.001**	0.036*	-	0.313	0.596	0.669
Day 5	0.022*	0.023*	0.247	0.313	-	0.756	0.795
Day 6	0.018*	0.017*	0.143	0.596	0.756	-	0.991
Day 7	0.015*	0.017*	0.170	0.669	0.795	0.991	-

According to the result from the test, differences in ETU levels between the days are significant for several of the days. The most substantial differences can be seen between the first two days and day 4. This result is also apparent in the graphs above where highest levels can be found day 1 and 2 and the lowest on day 4.

7.2.4.3 Difference in estimated exposure

Estimations were made upon the exposure to the children from the sprayings. The first approach was that there would be a direct exposure from the sprayings which would be represented by high ETU levels the day after spraying activity. Similarly if there was no spraying, there would be lower levels the day after. Since there was negligible spraying activities day 1, but the levels from day 2 show the

highest levels of the week, that approach was rejected. Instead it was assumed that after spraying activities, Mancozeb and ETU would be present in the environment on the spraying day and the day after. Mancozeb probably degrades quickly but since ETU is relatively stable to natural degradation it is assumed to stay in the environment for some time. Depending on where the spraying occurred and where the children were estimated to be during the spraying day and the day after, all the children were given an exposure grade (0; 1) every day, where 0 signifies no probable exposure and 1 represents probable exposure. Due to the new approach, all the children were supposed to be exposed day 1, since there were spraying activities near the school the day before. The only days where both exposure grades were present were day 1, 5, 6 and day 7. Mean, median and standard deviation of ETU levels for the two exposure groups these days are presented in table 10 together with the results from the Mann Whitney U test for significant differences between the groups.

Table 10. *The result from significance testing (Mann-Whitney U) of ETU levels depending on estimated exposure.*

Descriptive statistics and significance testing for the different exposure groups								
	Day 1		**Day 5**		**Day 6**		**Day 7**	
Median 0; 1	6.70	8.35	5.30	6.70	3.80	6.55	5.10	5.30
Mean 0; 1	7.06	9.10	5.75	7.08	4.32	6.57	5.66	6.21
Standard deviation 0; 1	3.63	4.61	2.83	2.40	1.92	3.87	2.42	4.01
Significance (Grouping factor exposure = 0; 1)	0.216		0.191		0.138		0.842	

No significant differences in the ETU levels due to estimated exposure were apparent in the results. Still, all means and medians are higher for exposure group 1 which indicates that the assumptions made on exposure grade are correct. Probably, more accurate observations on the children's estimated exposure would refine this result.

There is still no explanation why the ETU levels are so much higher day 1 and 2 than on day 5, 6 and 7, which all had sprayings on the previous days. One explanation could be that the heavy rain on day 3 washed the ETU off the ground. Whether or not there had been heavy rains before the field study begun is unknown. If not, there might have been an accumulation of ETU in the environment that would explain the higher levels on day 1 and day 2. Another explanation could be that the sprayings on day 0 occurred by the school and hence all the children would be exposed and therefore the mean of the ETU-level would increase.

7.2.4.4 Difference in ETU levels depending on distance of home to the plantation

The third factor assessed for a relationship with high ETU levels was the distance between the house where the children live and the banana plantation. Median values of the ETU levels were used in order to compensate for possible outliers within individual exposure. Figure 27 display the median levels with corresponding distances.

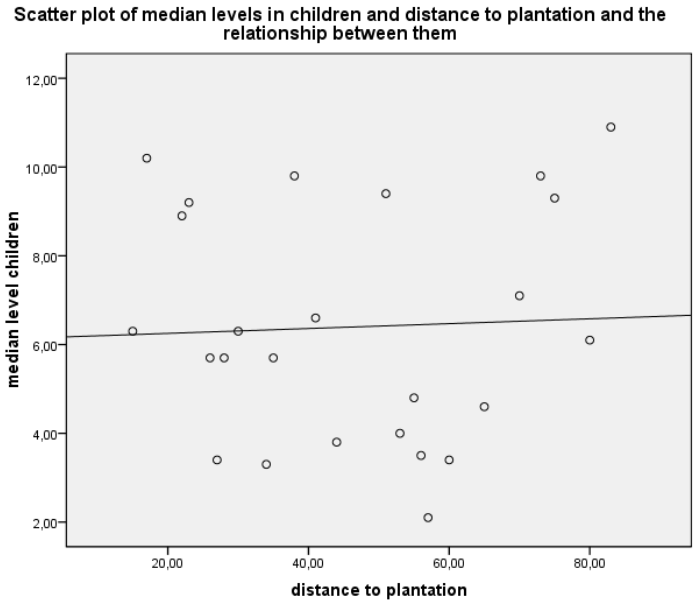

Figure 27. *A scatter plot of median ETU level in children corresponding to the distance between children's house and banana plantation.*

Spearman correlation test shows no significance in the correlation between children's ETU levels and the distance, which can also be seen in the figure. Because the mothers spent more time by their homes and their ETU were generally lower than the children's levels and not as fluctuating, an attempt in using the median of the ETU levels in the mothers and correlating that to the distances was done. This did, however, not show any significant correlation either. This result could be due to the shape of the village which is very narrow and hence the whole village is to some extent exposed when there are sprayings activities close by.

7.2.4.5 Difference in ETU levels depending on levels in parents and their occupation

There are significant higher ETU levels in fathers that work in the plantations compared to the ones that do not. However, when comparing the ETU levels in children with fathers occupation as grouping variable (1=work in field; 0=not work in field) there was not a significant difference between the two groups (p = 0,751 with Mann-Whitney U.). When comparing the mothers' levels with the fathers' occupation as grouping variable the 1-group (mean = 5.98) had significantly higher (p=0.001) ETU levels than the 0-group (mean = 3.72). This indicates that fathers to some extent bring exposure home, but the children are not as affected by it as the mothers. This could possibly be explained by more physical contact between the parents, or by other more important exposure factors among the children that might mask the correlation.

Further on median levels of fathers, mothers and children were tested for correlation with Spearman correlation test. The result is showed in Table 11.

Table 11. *Spearman correlation factors and significance for the correlation between median levels of ETU in fathers, mothers and children.*

		Child median	Mother median	Father median
Child median	Correlation coff.	1.000	-.012	.226
	Sig. (2-tailed)	.	.951	.268
	N	37	29	26
Mother median	Correlation coff.	-.012	1.000	.460*
	Sig. (2-tailed)	.951	.	.031
	N	29	29	22
Father median	Correlation coff.	.226	.460*	1.000
	Sig. (2-tailed)	.268	.031	.
	N	26	22	26

*. Correlation is significant at the 0.05 level (2-tailed).

As seen in table 11, there was no correlation between median ETU levels in fathers and children. Although there was a significant correlation between the levels of the mothers and the fathers which also can be seen in the scatter plot below (*Figure 28)*.

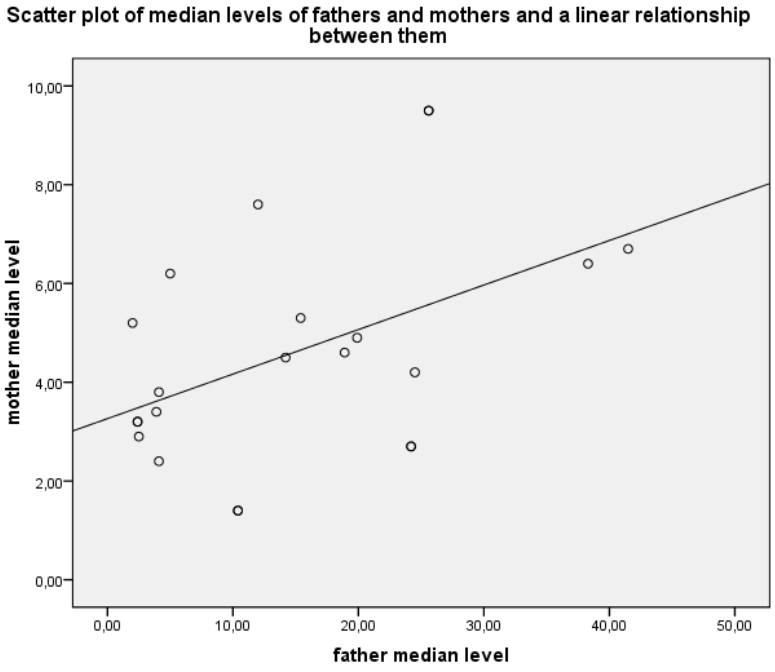

Figure 28. *A scatter plot on median levels of the mothers compared to median levels in their fathers.*

This result support the theory that the fathers to some extent bring exposure home and that the mothers are affected by it, but the same pattern is not true for the children. However, this does not mean that they do not get exposed by their fathers. As stated above it is probably due to other exposure factors that overshadow the exposure from the fathers.

7.2.4.6 Other factors of importance

Other factors that were tested to get a better understanding of the exposure situation for the children are presented below.

ETU levels in parents with occupation as grouping variable

As mentioned before, there was a significant difference in ETU level between fathers that work in the banana field and the ones that do not (p=0.000). The means of the different groups are presented in Table 12. There was an uncertainty whether the fathers in group 2 were working in the field or not, but according to the result, they seem not to.

Table 12. *The statistics for the fathers' different occupational groups.*

Descriptive statistics for fathers' levels depending on work			
Occ. Group	**Mean**	**Std. Dev.**	**N**
0	5.30	5.52	25
1	20.96	9.37	40
2	4.92	0.74	4

It is obvious that being in the field induces exposure to ETU/Mancozeb and lead to higher urinary ETU levels.

As presented before, mothers' levels are generally lower than the levels of the children and the fathers. This is assumed to be due to them staying inside a lot more than their children and the fathers. When comparing levels between mothers that work with packing the bananas they show significantly (p=0.002) lower levels (mean =2.88) than the mothers that are at home (mean= 5.18). This result is interesting but difficult to explain and will not be investigated any further.

Gender

Being a boy or a girl does not seem to play an important role in leading to high levels of ETU in the urine. There is not a significant difference between ETU-levels in boys and girls.

7.2.4.7 Summary and discussion of the statistical analysis

The results from the statistical analysis show that the most important factor for inducing high ETU levels in the children is the day of the collection. Results from the study by van Wendel de Joode et al (2008) showed higher levels after a spraying day, in comparison to a non-spraying day. However, that study was based on fewer measurements and uncertain spraying observations. The results from the present study, that is based on sufficient data and more certain spraying observations show that there were significantly higher levels after a day of spraying and two days after spraying, than a day, when there had not been spraying activities for three days and in addition heavy rains. From the spraying observations and the white paper method, we can conclude that it is very probable that Mancozeb and ETU drift into the village. Our conclusion is that Mancozeb is rather quickly degraded to ETU. ETU on the other hand is present in the environment for days after spraying activities. This is supported by chemical properties of the two substances (see Chapter 3). When the children play outside they get exposed from the ETU which is present in the environment if there have been sprayings on the days before. The evident differences between morning samples and spot samples show that some children have an uptake of ETU or Mancozeb between giving the morning sample and the spot sample. This implies that there is a more or less constant exposure in the environment, depending on how long ago there were aerial sprayings and if it had been raining or not. This constant exposure could be even more enhanced if the exposure is dermal which would lead to a slow uptake during a longer period of time.

The most probable exposure route in this case would probably be the skin, which has been mentioned as a possible route for ETU (Chapter 3.3); many of the children do not wear shoes when playing outside. Nevertheless, other exposure routes may be important as well. At the time of this study, a parallel exposure study was performed by a Berkley student Karen Phung. That study was based on a diary for the children, where they marked if they were indoors or outdoors and if they were playing actively or calmly. The result from that study were not finished at the time of writing this thesis, but it is possible that it will show higher levels for the children who were playing more outside than the ones that did not. Another result pointing in that direction is the lower ETU levels of the mothers who generally spent more time indoors than the children and the fathers.

Regarding the estimated exposure grade, the results are uncertain but the means are generally higher for the children who were estimated as exposed than the children who were estimated as non-exposed. However, observations are uncertain, and considering the amount of children that were under observation, we cannot be sure that children did not move into an area that had been close to the sprayings. It is also possible that the drift of pesticides from the aerial sprayings, more or less end up in every part of the village. This would lead to a more general exposure in the village than exposure just in different parts. This theory is strengthened by the fact that there is no correlation between ETU levels and the distance between houses and banana plantations.

The correlating ETU levels of the mothers and the fathers and the significant higher ETU levels in the mothers living with men who work in the plantations than the ones that do not, could indicate that fathers to some extent bring exposure home. However, none of these results were found in the children. As mentioned, this does not necessarily mean that they do not get exposed this way, but the result can be masked by other factors more important for children's exposure. Bringing pesticide exposure home is still regarded as a potential exposure factor.

One of the purposes with this thesis was to explain why some children have higher exposure than others. As can be seen, there were substantial differences in ETU levels between the children. However, with the observations done and with the results from this analysis, we are not able to explain that. Hopefully, results from the study by Karen Phung can explain some of the differences, but there can also be metabolic variations between the children that are difficult to model.

7.3 Risk characterization

Primarily the uncertainties originating from the assessment so far are presented and discussed. Thereafter the dose-response assessment and the exposure assessment are integrated and the risk is estimated.

7.3.1 Uncertainties in the risk assessment

In the problem formulation, the dose-response assessment and in the exposure assessment assumptions and extrapolations were made that are related to uncertainties. The data that have been collected and measured are also related to uncertainties. In this section the uncertainties mentioned in the previous chapters will be subject for a discussion. The outcome of the discussion will be whether or not the results still can be valid despite all the uncertainties.

7.3.1.1 Uncertainties related to the problem formulation

In the problem formulation, decisions about performance of the study were made and endpoints of the assessment were defined. The problem formulation in this study was an iterative process and the

decisions made in the beginning of the study were often subject for discussion. These were also changed throughout the process as new knowledge was gained. Because of this, the uncertainties regarding wrong focus or wrong endpoints are believed to be small. However, one major uncertainty that should be mentioned is the decision to only assess the risk for ETU and exclude Mancozeb. Consequently there might be risks associated to Mancozeb that are not accounted for in the risk assessment.

Exposure routes that were assessed excluded possible dietary exposure. No investigation of the children's food habits was conducted. This might possess an uncertainty.

In this study the risk assessment and the risk management are performed by the same persons. This may create a bias since risk assessment methods and data may be chosen to fit into the desired management. This was subject to a continuous discussion throughout all the process and hopefully avoided.

7.3.1.2 Uncertainties related to the dose-response assessment

The uncertainties from the dose-response assessment will be presented here in chronological order as the assessment was performed.

The data used to derive the regressions was found in the U.S. EPA's database for chemical toxicity information for aquatic and terrestrial life (www.epa.gov/ecotox) and from articles found for experimental studies on ETU.

The compared experiments have not always been conducted under the same conditions. For example, the route of exposure in the compared experiments could be a bit different. Furthermore, the endpoints were not always exactly the same in the compared experiment. Which experiments to use and not use have been up to the authors to evaluate and therefore it puts a degree of subjectivity to the model. Possible differences in the resulting model were not an object for examination. An attempt in overcoming this problem was to make rules to when experiments are too different to compare. For example the only exposure types that were included and considered similar enough were: *gavage, oral, diet* and *feeding*. Moreover, uncertainty in the input data was not quantified in this study. The ambition was to use studies that had been conducted under reliable conditions, with many test animals, several test doses and reliable results. Still the doses cannot considered as “true” NAELs and LAELs (No Adverse Effect Level/ Lowest Adverse Effect Level), since they only are doses in a predestinated interval.

Regressions were estimated in order to extrapolate from another chemical and to get a dose for NOAEL from a LOAEL. The uncertainties from these two extrapolations are derived in a similar way and will hence be discussed simultaneously.

The quantifiable uncertainties regarding the regressions were estimated by calculating a mean and standard deviation for the relationships. This was necessary since the regressions were by no means true linear relationships but rather approximations.

There were only a few values for each regression which made the result very sensitive for when including or when not including a specific value. For example, the gradient of the regression was extremely sensitive to if it included a LD50 or not. For the ETU/Malathion as well as for the NOAEL/LOAEL regression this uncertainty was partly overcome by using logarithmic values.

Malathion and Chlorpyrifos are both very different from ETU and they affect different species in different ways. Since the data material is rather small, one can suspect that there are effects for each pesticide that are not taken an account for in the distribution and hence the distributions might not describe reality well. Correlations were found for rat and mice, but that does not necessarily mean that other species, as assumed in the model, follow the same pattern. No further investigation of the toxicological properties of Malathion and Chlorpyrifos was conducted in this assessment.

The NOAEL/LOAEL regression was constructed with more data than the regressions between the different substances. It was also constructed with experimental data for additional animals and substances and can therefore be considered to be valid.

Another uncertainty was that the model was constructed of two serial regressions with corresponding equations. The outcome depended on which regression was used first which is expressed mathematically in the equations below.

$$y_1 = x * k_1 + m_1; \qquad y_2 = x * k_2 + m_2$$

$$Y_1 = (x * k_2 + m_2)k_1 + m_1 \Rightarrow Y_1 = xk_1k_2 + m_2k_1 + m_1$$

$$Y_2 = (x * k_1 + m_1)k_2 + m_2 \Rightarrow Y_2 = xk_1k_2 + m_1k_2 + m_2$$

Equation 1. *The equations show that the result is different depending on which equation that is used first, $Y_1 \neq Y_2$.*

Because the regression lines do not intersect the origin, the outcome will depend on which equation is aggregated into the other. In the model the equation which resulted in the lowest dose was chosen. This was thought to be a conservative decision but also the most correct. The doses were however not very different and two regressions were only made for cattle (ETU/Malathion, NOAEL/LOAEL) and for sheep (NOAEL/LOAEL, ETU/Chlorpyrifos).

As figure 29 shows, the uncertainty of the estimation magnifies when aggregating one distribution into another.

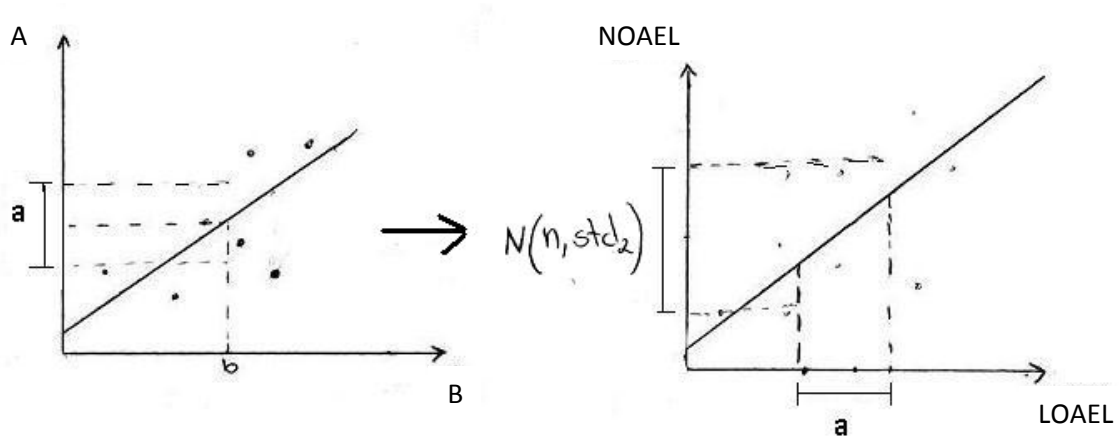

Figure 29. *The uncertainties from the first regression magnify the uncertainties of the estimation when aggregated in the second regression.*

Uncertainties regarding the ***difference in time duration*** are represented by assessment factors. When extrapolating between acute and chronic studies a uniform distribution for the safety factor

was applied. As mentioned in Chapter 6.2.3 the sensitivity for the final results was tested for this variable and it was not found to have a substantial impact on it.

Regarding the extrapolations from subacute and subchronic to chronic exposure a probabilistically derived assessment factor was used, that according to studies on the subject will cover 95 % of time difference extrapolations (Falk-Filipsson et al., 2007). This is a combination of a probabilistic and a conservative approach which was thought to be suitable for this study. Since the factors with this method have a scientific value and they are thought to be better estimations than the use of conventional safety factors.

Inter-species variations in sensitivity were represented with a SSD containing nine mammal species and the 1st percentile of this distribution was thought to correspond to a safe dose for 99 % of all mammal species. This approach was probabilistic although not very conservative.

Nine mammal species are not that many and it is not possible to guarantee that human's sensitivity is represented within this distribution. SSD is a method primarily used for ecological studies and not for human health risk assessments. The effects that the distribution is derived from are all effects that are measurable in experimental studies and there might be other more subtle effects lower doses. The differences in a species due to sensitive subgroups, such as children are not quantified with this model. There is a lack of knowledge about how chemicals interfere with developmental effects in unborn children, infants, children and adolescences.

Intra-species differences and sensitive subgroups should also be included in the model. A factor 15 was chosen based on recommendations from Falk-Filipsson et al. (2007). This is a number that is derived from several different studies on the subject and is both probabilistic and conservative. According to Falk-Filipsson et al. (2007) an extra assessment factor must be added if the risk assessment is performed for children and they recommend a factor from 1 to 10. For conservative reasons a factor 10 was chosen which make the overall assessment factor for both inter-individual differences and children's sensitivity to 150.

Since the goal of this study was to make a probabilistic, transparent risk assessment the last assessment factor of 10, which is not probabilistically derived, can be seen as misplaced. However it is our strongest believe that the extra assessment factor is needed for protecting the children. The reasons for this are that:

- ETU and Mancozeb have both showed to disturb development in animal studies
- There are few studies of that kind included in the model
- There were only nine mammals in the SSD
- The SSD is a model primarily used for ecological risk assessment and not for human health risk assessments

In this assessment **other Rfds** are also presented. They originate from U.S. EPA, IPCS and WHO. The Rfd from U.S. EPA is derived with the help of conservative assessment factors and the origin of the Rfds from IPCS and WHO are unknown.

To sum up; the uncertainties from the dose-response assessment are rather large. However the approach has been transparent and the uncertainties are presented. Also the dose-response

assessment does not only result in the Rfds assessed in this thesis but also include Rfds from three well known, trustworthy organizations that possess a lot of expert knowledge within the field.

7.3.1.3 Uncertainties related to the exposure assessment

In the following section different uncertainties associated with the exposure assessment will be discussed and explanations will be given for how they were treated. Many of the uncertainties cannot be quantified and will hence only be taken into the assessment through a qualitative discussion.

Suter (2007 p. 111) argues that only by looking at all chemicals present in a specific site and assess how they might affect each other can the true exposure and effects be assessed. In this study however, neither the time nor the resources were enough to look at any other chemicals than ETU and Mancozeb. Hence, there are considerable uncertainties regarding any additive, protective or synergic effects of other pesticides.

There are some uncertainties ***regarding the field study***. The children and their parents collected the urine themselves every morning and hence it is impossible to know whose urine was in a container and at what time it was collected. However many children, even if they did not have morning class, brought their samples in the morning which suggests that the collection was made that morning. Overall, regarding whose urine was in the container it was assumed that the families performed it correctly a majority of the time. Also, one sample that was suspected to be diluted with water was excluded from the study.

The week that the field study was performed was considered to represent a normal week in the village concerning spraying activities and the children's movement patterns. Still, our presence might have affected both the movement pattern of the children and the companies regarding their spraying schedule. However the latter is, by us, not thought to be probable, considering the extensive spraying activities during the week. The children's potential change in movement pattern might have changed the result of this study, but it is difficult to estimate in what direction. This week might also from other reasons have been a week of more or less exposure than the average. These are all uncertainties that only can be discussed and be used when designing future studies.

Although all efforts were put in to cover all sprayings some might have been missed because of the extension of the village. Since there were documented spraying activities every day except the days of rain it is not considered very likely that sprayings were missed to a large extent.

Samples were not frozen immediately after collecting. This method was approved by Lindh (Personal communication, July 2008) and hence not assumed to have changed the outcome of the analysis results. Uncertainties regarding the ***analytical method*** are discussed by Lindh et al. (2008).

When ***estimating the ETU levels to doses*** several estimations with associated uncertainties had to be made. To begin with the correlation between the excretion of the ETU and the oral dose that was used in the extrapolation was derived from only two test persons. Although the elimination half-life of the ETU was very similar for the two test persons it cannot be neglected that it was a very small experimental group and also the excretion rate from two adults was used to calculate the dose for children. The metabolism of children might differ much from that of adults and dilution of the urine

might not be the same. However, this was the only correlation that was found in the literature and was hence used.

The route of exposure was unknown although most probably primarily through lungs or skin in this study, whereas the only kind of exposure in Lindh et al.'s (2008) study was oral. This is a source of substantial uncertainty.

The timing for the exposure was also unknown. Looking at the ETU levels in the children in the village it is possible that there is more or less a constant exposure throughout the days. The exposure might also originate from various sources and through different exposure routes. An illustration of a possible exposure scenario in the village is shown in figure 30.

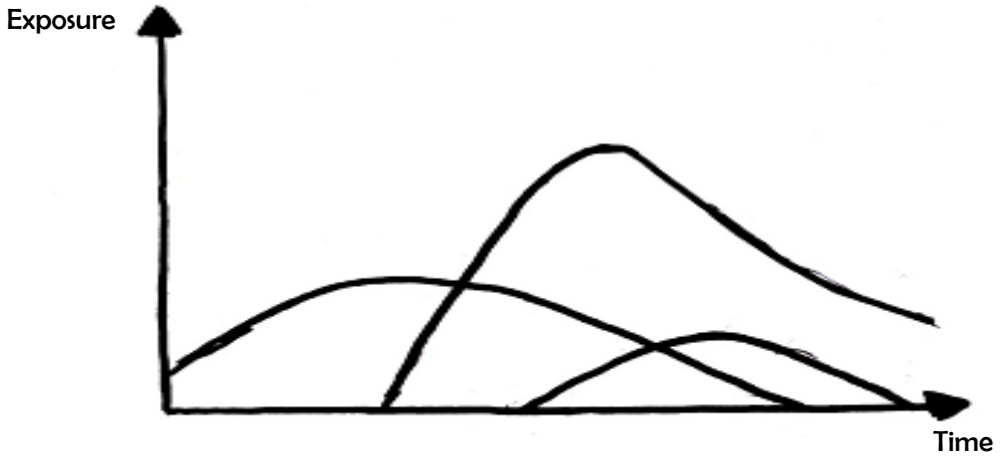

Figure 30. *The exposure may be from different exposure routes and may occur at different times. This contributes to a more or less constant exposure.*

To overcome all these uncertainties; metabolism differences, exposure routes differences and uncertainty in timing of exposure occasion, three different approaches were chosen as mentioned in Chapter 6.3.1.4. None of the doses that were calculated are believed to represent the reality but it is rather a method used in order to include uncertainties and variability.

The statistical analysis was performed to evaluate the factors that induce the exposure for the children. We are not statisticians and since the analysis was made to the best of our knowledge, there might exist better methods or better tools than were used. Medians were used instead of means in the correlations in order to avoid extreme values masking the result. Also the uncertainty regarding missing observations is a matter of concern for this part.

7.3.1.4 Other uncertainties

The children in the banana village are exposed to many more chemicals and pesticides than ETU and Mancozeb. Because there are signs of a correlation between the sprayings and the levels of ETU the children are most likely also exposed to the other chemicals in the cocktail. The previous study by van Wendel de Joode et al. (2008) also showed that there is exposure to other pesticides which are not applied by aerial spraying. No studies have been conducted on the probable synergistic, additive or antagonistic effects between these chemicals. Because of this it is very difficult to assess a true risk for the children.

7.3.2 Presentation of the results from the risk characterization

The oral doses calculated from the urinary ETU levels according to the three approaches presented in Chapter 6.3.1.4 were compared to the single potency values of U.S. EPA, FAO/WHO, IPCS and the one from our study.

In table 13, the percentage of children who had a dose above the Rfd is presented. The percentage of children who got a cancer risk above 10^{-6} is presented as well.

Table 13. *The Rfds and the ADIs from our study and from U.S. EPA, FAO/WHO and EU are presented and the percentage of children who will be above these doses depending on which approach is chosen*

	4 hours	12 hours	24 hours
U.S. EPA (2005)	43 %	94 %	100 %
FAO/WHO (1993)	0 %	0 %	6 %
IPCS (1993)	0 %	0 %	0 %
From this thesis	73 %	98 %	100 %
U.S. EPA (2005) cancer risk	100 %	100 %	100 %

As can be seen many of the children exceed the Rfds and all children are at risk for cancer according to U.S. EPA. This indicates that the children's exposure will lead to adverse effects. The cancer risk interval was between $2.05*10^{-6}$ - $1.87*10^{-4}$.

The most prominent factors to why the children are exposed to this extent are according to the statistical analysis in the exposure assessment:

- There are frequent aerial sprayings in the area
- Fathers who work in the plantations might bring exposure home

The uncertainties are many in this risk assessment. However many of them are quantified and hence present in the result and others have been a subject to discussion. We believe that the assessment is performed transparently and it should be possible for the reader to decide whether or not he/she believes that the result can still be used

8 Discussion regarding the risk treatment and risk communication

If choosing a right based criteria that none of the children should exceed any of the Rfds presented in this assessment, the risk is unacceptably high for the children in the village concerning ETU exposure. This chapter will discuss risk treatment alternatives that have been and can be proposed to reduce this risk. It will also discuss important aspects of risk communication regarding this specific subject.

8.1 Actions already proposed

The committee that was presented in Chapter 2.2.3 has decided upon trying to reduce exposure to children. However, process is slow and different interests in the group make collaboration difficult. During the meetings with the subcommittee some important goals have crystallized that van Wendel de Joode (Mail communication, November 2008) has provided to this study, these are:

- To communicate the result from this and the previous study to the ones that are responsible for spraying and to discuss how they think they could work in accordance with current legislation. They are waiting to set a date for such a meeting.
- To extend the current law that forbids spraying activities within 30 m distance to a houses in case of a natural barrier and 100 m without such a barrier, but does not regulate growing crops close to house (Reglamento para las actividades de la Aviación Agrícola, 2003). The proposal is to regulate growing crops as well.
- Actions should be taken at different levels, for example can legislation, timing of aerial spraying activities, education of local health representatives and the searching for alternatives be parallel treatment processes.

According to van Wendel de Joode (Mail communication, November 2008) everyone agrees on that they do not want the children to get ill from the spraying activities. Still, due to economic interests, it is hard to agree on actions to prevent that. She lists important actions the different stakeholders should take/are taking to make it possible to reduce the risk for the children.

- The University produces evidence for existing risk based on scientific research
- Ministry of Health (and other Ministries) adapts and controls legislation
- Certifiers intensifies revision to control that legislation is followed
- Companies follow legislation
- Villagers and teachers are empowered to be able to defend their rights to a clean environment

8.2 Discussion of proposed treatment actions

As mentioned before, all involved stakeholders agree on that they do not want the children to get ill from the pesticides. To have a common goal is of course a good thing. However, questions could be asked about how to reach this goal. In Chapter 4.2.1. risk treatment options are: decrease probability, decrease consequences or both. Decreasing probability actions in this case could be reducing exposure by for example reducing spraying activities, follow the current law and/or strengthen it. Wesseling et al. (2001) emphazise that the most effective way to reduce risk is to reduce pesticide use. Decreasing consequences could be treating sick children and strengthening the

families socioeconomic position. However, the result from this study is that the children are at risk for getting sick sometime in the future. It does not say anything about their health status as it is today. Treatment decisions in this specific matter, as we see it, should not be based on the latter but rather on possible consequences in the future. Wesseling et al. (2001) also stress that adverse health effects in the future are very likely to arise from today´s exposure. Nevertheless, strengthening the families' socioeconomic position, empower them to get an education and take responsibility for their health situation and surrounding environment are of course goals that should be aimed at as well. Wesseling et al. (2001) mention the need for strengthening workers' right to know and workers' empowerment; the academic sector should assist workers, high risk communities, and the general public with sound information. We suggest an integrated approach where both consequences and probability are diminished in parallel processes as suggested by the subcommittee.

The results from the assessment imply that being outdoors the following days after spraying activities causes high exposure to ETU. However, we want to accentuate that being outside and playing outside is a child's right. Actions based on regulating children's behavior are not recommended. The result also indicate that being in the plantation induce high exposure to ETU. It is known from the previous study that children frequently visit the plantation. Even in this case, our opinion is that it is the companies' responsibility to see to that children cannot enter the field. It is important that actions preventing that do not include threatening or sanctuary actions if the children still enter the field.

The current legislation as mentioned above forbids spraying activities within a distance of 30 m in case of a natural barrier, such as trees, and of 100 m without such a barrier. However, the law does not say anything about growing crops within this zone. Consequently, bananas are cultivated close to the village. Obviously it is more difficult controlling whether or not the companies are spraying within this buffer zone, than it would be controlling if there were crops there or not. According to observations from the present study it is evident that the current legislation is not followed. Further on, the results from this assessment show a clear correlation between spraying activities during the week and ETU levels. Thus, we can conclude that a law that regulates distance to aerial spraying is substantial to reduce exposure, and efforts should be focused on achieving control that it is followed. The banana companies should take their responsibility on the matter, governmental organs should see to that the law is followed, and certification organs (see Appendix A for more info about the standards for the two companies and what they require) should intensify their controls. Both companies are certified according to standards that have minimum requirement to follow the law.

The results of this thesis will not only be communicated to the stakeholders in the committee, but also to the ones that they concern the most, namely the families in the village. When humans are directly involved in a biomarker study like this it is important when reporting back the result to be sure not to cause additional psychological stress if the results show an exposure (NRC 1991 p.118). Even if there is a focus on risk reduction strategies at a higher level, it is important that villagers are informed about the results and what they can do to reduce the exposure. Wesseling et al. (2001) argue that research capacity should be applied to evaluate how cultural factors and risk perceptions affect the usefulness for different types of programs on pesticide use. In our opinion it is important in this specific case that misconceptions about exposure and pesticide related risks that were evident in Chapter 2.1.2 are made clear. Local risk reduction strategies should aim at supplying sound

information from non-profit organizations to the villagers about pesticide related risks and propose how individuals can reduce their risk.

Apart from the clear relationship between spraying activities and exposure, the statistical analysis also showed that the fathers probably bring exposure home to their families. This should be communicated to the families and strategies should be adopted in order to reduce this kind of exposure. We hold that it should be up to the companies to make it possible for them to shower and to change clothes before going home from work.

It is beyond the purpose of this study to analyze economic aspects of different risk treatment options, but it is obvious that economy has great impact of what actions that will be taken. Wesseling et al. (2001) argue that short-term economic interests overpower the policy discussions. The interests of export and those of the pesticide industry have overshadowed public health concerns (Wesseling et al., 2001). Clearly, economic interests are of great importance and as stated in Chapter 2.3.2, a great deal of the export revenues in Costa Rica is generated from the banana industry. The industry also employs a majority of the population in the region. We see the importance of finding solutions to the problem that are both economically and environmentally sustainable.

9 Conclusions

Depending on what value is used for the Rfd, the risk for the children can be characterized in different ways. None of the children are at risk when using the Rfd derived by IPCS (1993) and by using the Rfd estimated by FAO/WHO (1993) only a few percent are at risk, even with the most conservative approach for the exposure assessment. However, the other estimates for Rfd, the one estimated in this thesis and the one of U.S. EPA (2005) show that a majority of the children in the village are at risk. Depending on which approach that was chosen for the exposure assessment, 73.36 %, 98.18 % or 100 % of the children exceed the Rfd estimated in this thesis. Corresponding figures when using the Rfd of U.S. EPA would be 43.07 %, 94 % and 99.64 %. When estimating the cancer risk for the children by using the cancer potency factor that U.S. EPA (2005) estimated, 100 % of the children are at risk regardless to what approach is chosen. We can conclude that there is enough evidence to state that the ETU levels and the corresponding calculated doses are unacceptable for the children that live in the banana village. Considering the more or less constant exposure, there are reasons to believe that ETU can cause various adverse health effects in the children from a long term perspective. However, since these children are exposed to other pesticides in addition, there is an urge for more understanding of possible additive or synergetic factors of these.

The most distinct results from the statistical analysis show a clear correlation between aerial spraying activities and elevated ETU levels in the urine the days after. The result suggests that Mancozeb and ETU drift into the village and that ETU stays in the environment for a few days and causes exposure to the children who are playing outside. The lowest levels where found when there had not been spraying activities for three days and it had also been raining, which also indicate that the ETU is somewhat rinsed away with heavy rains. A conclusion can be that there is a more or less constant exposure in the environment, depending on how long ago there were aerial sprayings and if it has been raining or not. The current situation is that the aerial sprayings occur very close to the village and that there is an evident drift into the village. Our belief is that if the sprayings would occur further away, the exposure for the children would decrease. As suggested by the subcommittee, actions to reduce the risk should be taken at different levels, for example can legislation, timing of aerial spraying activities, education of local health representatives and the searching for alternatives be parallel treatment processes.

9.1 Future steps to take

There is an urge for more research in the study field. As for Mancozeb and ETU more data is needed regarding human uptake through different exposure routes and the excretion of ETU. A better understanding on how the urinary ETU levels correlate with a specific dose would improve the exposure assessment a lot . The results from this assessment would also be more reliable if there were information about Mancozeb/ETU content in the environment. More investigation about the persistence of the substances in the environment would be helpful. Also more research is needed concerning the effects in humans and specifically children, both regarding their development and other adverse effects when exposure is prolonged. As been stated before, concerning exposure to multiple chemicals in farm environment there is call for more knowledge regarding probable additive, synergic and antagonistic effects.

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10.2 Webpages

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10.3 Personal Communication

Bengtsson, G. Professor, Department of Ecology, Lund University, October 2008.

Lindh, C. Assistant Professor, Division of Occupational and Environmental Medicine, Lund University, July & November 2008.

Ruepert. C., El Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacionál, July 2008.

10.4 Mail Communication

Del Monte executive office (2008-09-30) *Certification information.*

Lindh, C. (2008-11-28), Assistant Professor, Division of Occupational and Environmental Medicine, Lund University, *Data from unpublished studies*.

Lindh, C. (2008-07-08), Assistant Professor, Division of Occupational and Environmental Medicine, Lund University, *Advises for the urine samples*.

Littorin, M. (2008-11-13), Senior Physician, Division of Occupational and Environmental Medicine, Lund University, *Uptake of Mancozeb and ETU.*

van Wendel de Joode, B (PhD). (2008-11-12), Profesora visitante, Instituto Regional de Estudios en Sustancias Tóxicas (IRET), Universidad Nacionál, *Stakeholder information.*

10.5 Figures

Figure 2

Costa Rica map: http://www.beachbumparadise.com/wp-content/uploads/2007/06/costa-rica-map.jpg

Central America map: http://schema-root.org/region/americas/central_america/central_america.jpg

Banana village map: Córdoba Gambóa, L. (Geographer) (2008)

INFO ABOUT THE STANDARDS THAT THE BANANA COMPANIES ARE CERTIFIED WITH

CHIQUITA

Chiquita is certified by rain forest alliance which is divided in ten principles. Each principle has several criterions that should be fulfilled by the certified farm. To keep the certification 50 % of the criterions within each principle and 80 % of all criterions must be fulfilled. There are also some critical criterions which the farm must be completely complied with in order to keep or get the certification. One critical criterion to keep certification is that no substances that are forbidden by U.S. EPA or the European Union can be used in the farm (SAN standard Feb. 2008). According to the sustainable agriculture standard (www.rainforest-alliance.com) the distance between areas of crop production must be at a distance of 30 m from houses or according to the legislation of the country whatever is the stricter.

BANDECO

Del Monte plantations in Costa Rica are certified in accordance with Global Gap (formerly known as Eurep GAP), SA-8000, Social Accountability, ISO 9001 (Quality Management Systems), ISO 14000 (Environmental Management Systems, OHSAS 18001 (Occupation Health and Safety) (Del Monte US executive office, Mail communication). Del Monte states on its webpage that they do not use products banned by the United States, European Union, or World Health (www.freshdelmonte.com)

OHSAS 18001 is a certification which aims to help an organization to control the occupational health and safety risks (www.ohsas-18001-occupational-health-and-safety.com). One of the benefits with the system presented on the OHSAS 18001 webpage is that it can help to eliminate or minimize the risks to employees and other interested parties.

SA-8000 is focusing on workers right and has been developed by SAI (Social Accountability International), that is a non-profit affiliate of the Council on Economic Priorities (CEP) (www.mallenbaker.net; www.sa-intl.org). It is based on the principles of human rights norms as described in International Labor Organization conventions, the United Nations Convention on the Rights of the Child and the Universal Declaration of Human Rights (www.mallenbaker.net). It focuses on eight key areas: child labor, forced labor, health and safety, free association and collective bargaining, discrimination, disciplinary practices, working hours and compensation (SAI, 2001). According to SAI (www.sa-intl.org) itself it is one of the strongest voluntary standards that exist. The criterions of interest are that workers must be guaranteed health care and that the company shall not expose children or young workers to situations in or outside of the workplace that are hazardous, unsafe, or unhealthy (SAI, 2001).

ISO 9001 specifies requirements for a quality management system.

Certification by the ISO 14000 family specifies requirements for an environmental management system to enable an organization to develop and implement a policy and objectives which take into account legal requirements and other requirements to which the organization subscribes, and information about significant environmental aspects. It applies to those environmental aspects that the organization identifies as those which it can control and those which it can influence.

Global GAP, where GAP stands for good agricultural practice, which is primarily designed to reassure consumers about how food is produced on the farm by minimizing detrimental environmental

impacts of farming operations, reducing the use of chemical inputs and ensuring a responsible approach to workers´ health and safety as well as animal welfare (www.globalgap.org). Local regulations should be checked first of all to verify legal compliance

TABLE OVER STUDIED EXPERIMENTS ON MANCOZEB AND ETU-1

Table A. *The table shows the experimental studies that have been examined in this thesis, some of them are included in the dose-response assessment, and some constitute material for an overall toxicity judgment.*

Article	Test animal	Effect studied
Exposure to ETU		
Graham et al. (1975)	Charles River rat	Increased incidence of thyroid hyperplasia.
Chhabra et al. (1992)	Inbred Fisher rat	Hormonal alterations increased weight of liver and thyroid glands.
U.S. EPA (1996) refers to Graham and Hansen (1972)	Osborne Mendel rat	Thyroid hyperplasia.
FAO/WHO (1993) refers to unpublished study by Rohm and Haas companies	Sprague Dawley rat	Thyroid hormonal changes, increased liver and thyroid weight.
U.S. EPA (1996) refers to Freudenthal (1977)	Sprague Dawley rat	Thyroid hyperplasia.
FAO/WHO (1993) refers to unpublished study by Rohm and Haas companies	Sprauge Dawley rat	Thyroid gland follicular cell hyperplasia and hypertrophy.
Kurttio et al. (1986)	Wistar rat Male	Inhibited dose-dependently T4- and T3-secretion.
U.S. EPA (1996), FAO/WHO (1993) refers to an unpublished study by Rohm and Haas companies	Mouse male	Increased thyroid hyperplasia and liver weight.
U.S. EPA (1996), FAO/WHO (1993) refers to an unpublished study by Rohm and Haas companies	Mouse female	Increased thyroid hyperplasia and liver weight.
Khera & Iverson (1978)	Cat	Loss of body weight, tremors.
FAO/WHO (1993) and U.S. EPA (1996) refers to unpublished study by Rohm and Haas companies	Beagle dog	Decreased hemoglobin.
FAO/WHO (1993) and U.S. EPA (1996) refers to unpublished study by Rohm and Haas companies	Beagle dog	Reduction of body weight gain, hypertrophy of the thyroid with colloid retention. Increased thyroid weight.
Teratogenicity		
Saillenfait et al (1991)	Sprauge Dawley rat	Dilated brain cavities.
Khera et al. (1973)	Rat	Various lesions in fetus
Khera et al. (1973)	Rabbit	Decreased brain weight in fetus.
FAO/WHO (1993) refer to Teramoto (1978)	Wistar-Imamichi rats	Dilated brain cavities.
FAO/WHO (1993) refer to Teramoto (1978)	Hamster	Decrease of fetal bodyweight.
Exposure to Mancozeb		
Mahadevaswami et al. (1999)	Albino rat	Impairment of the estrogen cycle.
Baligar & Kaliwar (2001)	Wistar Starin rat	Impairment of the estrogen cycle.

TABLE OVER STUDIED EXPERIMENTS ON MANCOZEB AND ETU-2

Table B. *The table shows the experimental studies that have been examined in this thesis, some of them are included in the dose-response assessment, and some constitute material for an overall toxicity judgment.*

Cancer			
Belpoggi (2002) (Mancozeb)	Sprague Dawley rat	Increased total malignant tumors in all Mancozeb-treated groups. In males increase was dose related.	
Graham et al. (1975)	Charles River rat	Cancer appeared at 125 ppm but was obvious at 250 ppm.	
Other studies			
Chhabra et al. (1992)	Inbread Fisher Rat pup	ETU in vivo perinatal, adult and combined feeding study	Hormonal alterations increased weight of liver and thyroid glands at the dose of 6,2mg/kg/day
Cecconi et al. (2006) refer to Greenlee et al.	Two cells' mouse embryo	Mancozeb in vitro	Dramatically impairment of development of embryo
Mehrotra et al. (1987)	Mouse	Mancozeb in vivo study with application on skin.	A Tumour initiating ability of Mancozeb in mouse skin was found.
Leiphon & Picklo (2006)	Rat brain mitochondria	Mancozeb and Maneb in vitro study	Inhibition of enzymes in the mitochondria which possibly can lead Parkinson.
Chhabra et al. (1992); U.S. EPA (1996)	Mouse	ETU in vivo perinatal, adult and combined feeding study	Hormonal alterations increased weight of liver and thyroid glands
Rossi et al. (2006)	Mouse	Mancozeb in vivo teratogenic feeding study	Alterations at the early phase of reproduction and fetus development.
FAO/WHO (1993) refer to an unpublished study by Rohm and Haas by Rohm and Haas companies	Sprague Dawley rats	ETU in vivo feeding study	Highest dose tested was NOAEL
Saillenfait et al., (1991)	Sprauge Dawley rat	ETU in vivo feeding study	Highest dose tested was NOAEL

ASSESSING RFD FOR ETU ACCORDING TO U.S. EPA

When U.S. EPA (1996) calculated a Rfd for oral exposure for ETU they added a safety factor of 100 to cover for intra- and interspecies differences, a factor of 3 to cover for limited data on developmental and multi generation data and 10 for extrapolation from LOAEL to NOAEL. This reference dose was derived from one study of rats and confirmed in other studies. All together the total uncertainty factor was 3000. In a later attempt from U.S. EPA (2005 p. 16-18) to estimate a Rfd for ETU a uncertainty factor of 100 was determined for inter and intra species differences and an uncertainty factor of 10 for the lack of developmental neurotoxicity studies was added as well. This reference dose was derived from a study on dogs (U.S. EPA, 2005 p.17). Table A shows the results from U.S. EPA's most resent Dose-Response assessment of ETU. A similar Table for Mancozeb is found in table B.

Table A. *Toxicological endpoints for ETU, according to U.S. EPA (2005).*

Exposure Scenario	Dose	Study and type of effect
Dietary Exposure		
Acute, females age 13-49	NOAEL=5 mg/kg/day Acute RfD= 0,005 mg/kg/day	Developmental Toxicity study on rats. Based on developmental defects in the brain.
Chronic, general population	NOAEL=0,18 mg/kg/day Chronic RfD = 0,0002 mg/kg/day	Chronic toxicity study on dogs. Based on thyroid toxicity
Dermal Exposure		
Long-Term (> 6 months)	NOAEL = 0,18/kg/day	Chronic toxicity study on dogs. Based on thyroid toxicity
Inhalation Exposure		
Long-Term (> 6 months)	NOAEL = 0,18/kg/day	Chronic toxicity study on dogs. Based on thyroid toxicity

Table B. *Toxicological endpoints for Mancozeb, according to U.S. EPA (2005) presented.*

Exposure Scenario	Dose	Study and type of effect
Dietary Exposure		
Acute, females age 13-49	NOAEL=128 mg/kg/day	Developmental Toxicity study on rats Based on hydrocephaly and other malformations
Chronic, general population	NOAEL=4,83 mg/kg/day Chronic Rfd = 0,05 mg/kg/day	Toxicity/Carcinogenicity study on rats Based on thyroid toxicity
Dermal Exposure		
Long-Term (> 6 months)	NOAEL = 4,83 mg/kg/day	Toxicity/Carcinogenicity study on rats Based on thyroid toxicity
Inhalation Exposure		
Any duration	NOAEL = 0,079 mg/L	Subchronic Inhalation study on rats Based on thyroid hyperplasia and decreased thyroxine

TABLE OF URINE COLLECTION

Table A. *The urine collection of the children*

	Gender	Years in village	Distance to field	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dayt 60	**boy**	7	28	1	1	1	1	1	1	1
Dayt 49	**girl**	4	22	0	0	1	1	1	1	1
Dayt 18	**girl**	9	22	1	1	1	7	1	1	1
Dayt 1	**boy**	9	22	1	1	1	7	1	1	1
Dayt 17	**boy**	8	70	1	1	1	1	1	1	1
Dayt 20	**girl**	4	55	1	1	1	1	1	1	1
Dayt 16	**girl**	9	26	1	1	1	1	1	1	1
Dayt 61	**boy**	4	35	1	1	1	1	1	6	1
Dayt 47	**girl**	9	23	1	1	1	1	1	1	1
Dayt 57	**girl**	5	51	1	1	1	1	1	1	1
Dayt 31	**boy**	8	15	1	1	1	1	1	1	1
Dayt 56	**boy**	10	60	2	1	1	1	1	1	1
Dayt 34	**girl**	8	34	3	2	3	1	5	1	1
Dayt 36	**boy**	3	30	1	3	1	4	4	0	2
Dayt 54	**girl**	3	30	1	3	1	4	4	0	2
Dayt 3	**girl**	8	53	7	1	1	1	1	1	2
Dayt 43	**girl**	6	56	1	1	1	1	1	1	1
Dayt 48	**boy**	6	56	1	1	1	1	1	4	1
Dayt 45	**girl**	9	57	1	1	1	1	1	1	1
Dayt 24	**boy**	9	27	1	1	1	1	1	1	1
Dayt 27	**boy**	9	41	1	1	1	1	1	1	1
Dayt 29	**boy**	9	17	1	1	1	1	1	1	1
Dayt 39	**girl**	-	65	1	1	3	1	1	1	1
Dayt 32	**boy**	-	65	1	1	3	1	1	1	1
Dayt 53	**girl**	10	80	1	1	1	1	1	1	1
Dayt 41	**girl**	10	75	1	2	1	1	1	7	1
Dayt 42	**boy**	8	55	1	1	1	1	1	1	1
Dayt 59	**girl**	8	75	1	1	1	1	1	1	1
Dayt 10	**boy**	7	73	4	1	1	1	2	1	1
Dayt 5	**girl**	9	70	0	1	2	1	1	1	1
Dayt 6	**boy**	5	38	1	1	1	1	2	1	1
Dayt 7	**girl**	8	83	1	1	1	1	1	1	1
Dayt 8	**boy**	8	41	1	1	1	1	1	1	1
Dayt 40	**boy**	8	44	1	1	1	1	4	1	1
Dayt 62	**boy**	9	30	1	1	1	1	3	4	1
Dayt 30	**boy**	8	30	1	1	1	1	1	1	1
Dayt 52	**girl**	10	30	1	1	1	1	1	1	1

Codes: 1= received sample, **0**= no received sample, **2**= received sample in the morning, but not the first urine, **3**=received sample from lunch time, **4**= received sample from afternoon, **5**= received sample from the night before, **6**=unsure on from when the sample is taken **7**=small amount of urine, perhaps the second urine in the morning

TABLE OF EXPOSURE ESTIMATION FOR ALL CHILDREN ALL DAYS

Table B. *Presentation of the exposure grade of the children. Exposure of day 1 corresponds to urine sample day 1 but the estimated exposure is for two days before the collection day.*

	Gender	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dayt 60	**boy**	0	1	0	0	0	0	0
Dayt 49	**girl**	-	-	0	0	0	1	1
Dayt 18	**girl**	1	1	0	0	0	1	1
Dayt 1	**boy**	0	1	0	0	0	1	1
Dayt 17	**boy**	1	1	0	0	0	1	1
Dayt 20	**girl**	1	1	0	0	0	1	1
Dayt 16	**girl**	1	1	0	0	0	0	0
Dayt 61	**boy**	0	1	0	0	0	0	0
Dayt 47	**girl**	0	1	0	0	0	1	0
Dayt 57	**girl**	0	1	0	0	0	0	0
Dayt 31	**boy**	1	1	0	0	1	1	0
Dayt 56	**boy**	1	1	0	0	0	0	0
Dayt 34	**girl**	0	1	0	0	1	1	1
Dayt 36	**boy**	1	1	0	0	1	-	1
Dayt 54	**girl**	1	1	0	0	-	-	1
Dayt 3	**girl**	1	1	0	0	0	1	1
Dayt 43	**girl**	0	1	0	0	0	1	1
Dayt 48	**boy**	0	1	0	0	0	1	1
Dayt 45	**girl**	0	1	0	0	0	1	1
Dayt 24	**boy**	1	1	0	0	0	1	1
Dayt 27	**boy**	1	1	0	0	0	1	1
Dayt 29	**boy**	1	1	0	0	0	1	1
Dayt 39	**girl**	1	1	0	0	0	1	1
Dayt 32	**boy**	1	1	0	0	1	1	0
Dayt 53	**girl**	1	1	0	0	0	0	1
Dayt 41	**girl**	1	1	0	0	0	1	1
Dayt 42	**boy**	1	1	0	0	0	1	1
Dayt 59	**girl**	0	1	0	0	0	0	1
Dayt 10	**boy**	1	1	0	0	0	1	1
Dayt 5	**girl**	-	1	0	0	0	1	1
Dayt 6	**boy**	0	1	0	0	0	1	1
Dayt 7	**girl**	1	1	0	0	0	1	1
Dayt 8	**boy**	1	1	0	0	0	1	1
Dayt 40	**boy**	0	1	0	0	0	0	0
Dayt 62	**boy**	1	1	0	0	0	0	1
Dayt 30	**boy**	0	1	0	0	1	1	1
Dayt 52	**girl**	1	1	0	0	1	1	1

Codes: 0= children estimated to not be exposed, **1**= children estimated to be exposed. - =no urine sample.

TABLE OVER URINE COLLECTION AND QUESTIONNAIRE FREQUENCY FOR PARENTS-1

Table C1. *Data for the urine collection and questionnaire for parents.*

	day 1	day 2	day 3	day 4	Questionnai
Dayt 60 mother	1	1	1	1	1
Dayt 60 father	-	-	-	-	-
Dayt 49 mother	0	0	0	1	1
Dayt 49 father	0	0	0	0	1
Dayt 18 & 1 mother	1	1	1	1	1
Dayt 18 & 1 father	1	1	1	1	1
Dayt 17 mother	1	1	1	0	1
Dayt 17 father	0	1	1	1	1
Dayt 20 mother	0	0	0	0	0
Dayt 20 father	1	1	1	1	1
Dayt 16 mother	1	1	1	1	1
Dayt 16 father	1	1	1	1	1
Dayt 61 mother	1	1	1	1	1
Dayt 61 father	1	1	1	1	1
Dayt 47 mother	1	1	1	1	1
Dayt 47 father	1	1	1	1	1
Dayt 57 mother	1	1	1	1	1
Dayt 57 father	1	1	1	1	1
Dayt 31 mother	1	1	1	1	1
Dayt 31 father	1	1	1	1	1
Dayt 56 mother	0	1	1	1	1
Dayt 56 father	0	1	1	1	1
Dayt 34 mother	-	-	-	-	-
Dayt 34 father	2	1	0	1	1
Dayt 36 & 54 mother	0	0	0	0	1
Dayt 36 & 54 father	0	0	1	0	1
Dayt 3 mother	0	1	1	1	1
Dayt 3 father	0	1	1	1	1
Dayt 43 & 48 mother	1	1	1	1	1
Dayt 43 & 48 father	1	1	1	1	1
Dayt 45 mother	0	1	1	1	1
Dayt 45 father	-	-	-	-	1
Dayt 24 mother	1	1	1	1	1
Dayt 24 father	0	1	1	1	1
Dayt 27 mother	1	1	1	1	1
Dayt 27 father	1	1	1	1	1
Dayt 29 mother	1	1	1	1	1
Dayt 29 father	1	1	1	0	1
Dayt 39 & 32 mother	1	1	0	0	0
Dayt 39 & 32 mother	1	1	0	0	0

Codes: 0= sample missing, **1**= received sample, first morning void, **2**= received sample from lunch time, **3**= received sample but very light, - = no participation

TABLE OVER URINE COLLECTION AND QUESTIONNAIRE FREQUENCY FOR PARENTS-2

Table C2. *Urine collection for the parents and partition with the questionnaire*

	day 1	day 2	day 3	day 4	Questionnaire
Dayt 53 mother	1	1	1	1	1
Dayt 53 father	-	-	-	-	1
Dayt 41 mother	1	0	1	1	1
Dayt 41 father	1	0	1	1	1
Dayt 42 mother	-	-	-	-	0
Dayt 42 father	0	1	1	1	1
Dayt 59 mother	1	1	1	1	1
Dayt 59 father	-	-	-	-	-
Dayt 10 mother	0	1	1	0	1
Dayt 10 father	0	0	1	0	1
Dayt 5 mother	0	1	0	0	1
Dayt 5 father	-	-	-	-	1
Dayt 7 mother	3	0	0	3	1
Dayt 7 father	0	3	3	3	1
Dayt 8 mother	3	3	3	3	1
Dayt 8 father	-	-	-	-	1
Dayt 62 mother	1	1	1	1	1
Dayt 62 father	-	-	-	-	-
Dayt 30 & 52 mother	1	1	1	1	1
Dayt 30 & 52 father	1	1	1	1	1
Dayt 6 mother	-	-	-	-	-
Dayt 6 father	-	-	-	-	1
Dayt 40 mother	-	-	-	-	1
Dayt 40 father	-	-	-	-	1

Codes: **0**= sample missing, **1**= received sample, first morning void, **2**= received sample from lunch time, **3**= received sample but very light, - = no participation

TABLE OVER ANSWER FROM THE QUESTIONNAIRES- 1

Table D1. *Answers from the questionnaires. Which company owns their house, years they have lived in the banana village, what kind of work they do, where they wash their clothes, where they keep their boots and time duration from spraying until they are let in to the plantations.*

	House	years in Daytonia	Work	working clothes	Washing	boots	time duration
Dayt 60 mother	-	25	0	0	0	0	0
Dayt 49 mother	1	4	0	0	0	0	0
Dayt 49 father	1	4	1	1	1	1	1
Dayt 18 & 1 mother	1	10	0	0	0	0	0
Dayt 18 & 1 father	1	10	1	2	2	1	1
Dayt 17 mother	1	13	0	0	0	0	0
Dayt 17 father	1	13	1	2	2	1	0
Dayt 20 father	1	4	1	1	1	1	1
Dayt 16 mother	1	16	0	0	0	0	0
Dayt 16 father	1	16	0	1	1	1	6
Dayt 61 mother	1	4	0	0	0	0	0
Dayt 61 father	1	10	1	2	2	1	3
Dayt 47 mother	0	9	0	0	0	0	0
Dayt 47 father	0	9	0	0	0	0	0
Dayt 57 mother	1	5	0	0	0	0	0
Dayt 57 father	1	15	0	1	1	1	6
Dayt 31 mother	1	18	1	1	1	1	6
Dayt 31 father	1	11	2	1	1	1	1
Dayt 56 mother	1	10	0	0	0	0	0
Dayt 56 father	1	10	0	1	1	1	0
Dayt 34 father	2	4	1	1	1	1	2
Dayt 36 & 54 mother	2	3	0	0	0	0	0
Dayt 36 & 54 father	2	4	1	1	1	1	2
Dayt 3 mother	2	15	1	1	1	1	2
Dayt 3 father	2	15	1	1	1	1	2
Dayt 43 & 48 mother	2	6	0	0	0	0	0
Dayt 43 & 48 father	2	6	1	1	1	1	2

Storing boots, washing clothes, working clothes: 0 = do not work in the company, **1 =** at home/their own, **2=** the company does it, **House: 1**= Del Monte, **2=** Chiquita

Work

father

0=do not work in field

1=work in field

2=unsure if in field or not

mother

0=do not work in the company

1=work in the packing facilities

Time duration

0= Do not work in the company

1=1 hour

2=the next day

3= < 1 hour

4= 1-4 hours

5= > 4 hours

6= I do not enter

TABLE OVER ANSWER FROM THE QUESTIONNAIRES -2

Table D2. *Answers from the questionnaires. Which company owns their house, years they have lived in the banana village, what kind of work they do, where they wash their clothes, where they keep their boots and time duration from spraying until they are let in to the plantations.*

	House	years in Daytonia	work	working clothes	Washing	boots	time duration
Dayt 45 mother	2	10	0	0	0	0	0
Dayt 45 father	2	10	1	2	2	1	2
Dayt 24 mother	2	15	0	0	0	0	0
Dayt 24 father	2	15	1	1	1	1	0
Dayt 27 mother	2	12	0	0	0	0	0
Dayt 27 father	2	15	1	1	1	1	6
Dayt 29 mother	2	9	0	0	0	0	0
Dayt 29 father	2	-	1	2	2	1	2
Dayt 53 mother	1	15	0	0	0	0	0
Dayt 53 father	1	10	1	1	1	1	3
Dayt 41 mother	1	13	0	0	0	0	0
Dayt 41 father	1	13	1	1	1	1	1
Dayt 42 father	2	8	1	1	1	1	6
Dayt 59 mother	2	30	1	1	1	1	6
Dayt 10 mother	1	7	0	0	0	0	0
Dayt 10 father	1	7	1	1	1	1	5
Dayt 5 mother	1	19	0	0	0	0	0
Dayt 5 father	1	19	1	1	1	1	4
Dayt 7 mother	1	15	0	0	0	0	0
Dayt 7 father	1	15	1	2	2	1	4
Dayt 8 mother	1	18	0	0	0	0	0
Dayt 8 father	1	18	1	2	2	1	1
Dayt 62 mother	2	14	1	1	1	1	6
Dayt 30 & 52 mother	2	6	0	0	0	0	0
Dayt 30 & 52 father	2	30	0	1	1	1	6
Dayt 6 father	1	5	1	2	1	1	0
Dayt 40 mother	2	10	0	0	0	0	0
Dayt 40 father	2	10	1	1	1	1	2

Storing boots, washing clothes, working clothes: 0= do not work in the company, **1=** at home/their own, **2=** the company does it, **House: 1**= Del Monte, **2=** Chiquita

Work

father

0=do not work in field

1=work in field

2=unsure if in field or not

mother

0=do not work in the company

1=work in the packing facilities

Time duration

0= Do not work in the company

1=1 hour

2=the next day

3= < 1 hour

4= 1-4 hours

5= > 4 hours

6= I do not enter

TABLES OVER EXPERIMENTAL DATA USED IN THE DOSE-RESPONSE ASSESMENT-1

Table A. *The experimental studies on ETU used in the dose-response assessment. The data is taken from the experimental studies presented in Appendix B.*

species	endpoint	dose	unit	effect type	effect	effect site	time -	exposure type	Reference
Rat	LD50	**1832**	mg/kg	mor	gain	Not Reported	not sure	Oral	U.S. EPA (1996) refr. Graham & Hansen (1972)
Rat	LOAEL	**4,1**	mg/kg	horm, phy, inj	gain	organ	24 month	Feeding	Chhabra et al. (1992)
Rat	NOAEL	**0,37**	mg/kg	horm, phy, inj	gain	organ	24 month	Feeding	U.S. EPA (1996) refr. Rohm & Haas
Mouse	LOAEL	**17,2**	mg/kg	morf	smix	liver	24 moths	Feeding	U.S. EPA (1996) refr. Rohm & Haas
Mouse	NOAEL	**1,72**	mg/kg	morf	smix	liver	24 month	Feeding	U.S. EPA (1996) refr. Rohm & Haas
Rat	LOAEL	**20**	mg/kg	tera	morf	brain	20 days	Feeding	Teramoto (1978)
Rat	NOAEL	**10**	mg/kg	tera	morf	brain	20 Days	Feeding	Teramoto (1978)
Mouse	LD50	**4000**	mg/kg	mort	mort	Not Reported	not sure	Oral	Lewerentz & Plass (1984)

Table B *The experimental studies on Chlorpyrifos used in the dose-response assessment. The data is taken from U.S. EPAs database for chemical toxicity information for aquatic and terrestrial life (www.epa.gov/ecotox)*

Species	endpoint	dose	unit	effect type	effect	effect site	time (days)	exposure type	Ref.nr
Rat	LD50	**169**	mg/kg org	MOR	MORT	Not Reported	14	Gavage	35039
Rat	LOAEL	**1**	mg/kg/d	ENZ	CEST	Plasma	133	Gavage	93040
Rat	NOAEL	**0,1**	mg/kg/d	ENZ	CEST	Plasma	133	Gavage	93040
Rat	LOAEL	**25**	mg/kg/d	DVP	ABNM	Palate	16	Gavage	92585
Rat	NOAEL	**15**	mg/kg/d	DVP	ABNM	Palate	16	Gavage	92585
Mouse	LD50	**152**	mg/kg org	MOR	MORT	Not Reported	14	Gavage	35039

TABLES OVER EXPERIMENTAL DATA USED IN THE DOSE-RESPONSE ASSESSMENT-2

Table C. *The experimental studies on Malathion used in the dose-response assessment. The data is taken from U.S. EPAs database for chemical toxicity information for aquatic and terrestrial life (www.epa.gov/ecotox)*

Species	endpoint	dose	unit	effect type	effect	effect site	time (days)	exposure type	Ref.nr
rat	LD50	331.2	mg/kg	mor	mort	Not reported	4	gavage	90649
rat	LOAEL	24	ppm	mph	weight	Liver	106.54	feed	89875
rat	NOAEL	1.6	ppm	mph	weight	liver	106.54	feed	89875
mouse	LOAEL	100	ppm	mph	smix	spleen	56	diet	90630
mouse	NOAEL	50	ppm	mph	smix	spleen	56	Diet	90630
mouse	LD50	934	mg/kg	mor	mort	Not reported	7 days	Oral	6905

Table C. *The experimental studies on ETU used in the dose-response assessment for NOAEL/LOAEL extrapolation. The data is taken from U.S. EPAs database for chemical toxicity information for aquatic and terrestrial life (www.epa.gov/ecotox)*

Species	Substance	NOAEL dose	LOAEL dose	unit	effect type	effect	effect site	time (days)	exposure type	Ref.
mouse	ETU	300	600	mg/kg	gro	wght	body	8	gavage	90438
mouse	ETU	357	1006	ppm	mph	wght	li	273.96	diet	90086
rat	ETU	0.37	4.1	mg/kg	horm, phy, inj	gain	organ	720	feeding	U.S. EPA (1996) refr. Rohm & Haas
mouse	ETU	1.72	17.2	mg/kg	morf	smix	liver	720	feeding	U.S. EPA (1996) refr. Rohm & Haas
rat	ETU	40	80	mg/kg	mort	mor	-	7	feeding	Khera et al. (1973)
Hamster	ETU	600	1200	mg/kg	gro	wght	-	4	gavage	90448
black rat	carbaryl	125	250	mg/ kg	hrm	ghrm	sr	6.33	gavage	87551
goat	malathion	75	150	mg/ kg	enz	asat	sr	1	diet	40239
mouse	malathion	0.1	10	mg/ kg	enz	genz	prt	0.167	gavage	90632
mouse	malathion	50	100	ppm	mph	smix	sp	56	diet	90630
rat	malathion	0.118	1.776	ppm	enz	ache	br	106.54	feed	90627
rat	malathion	55	137.5	mg/ kg	enz	alph	br	32	gavage	88916
rat	malathion	5	50	mg/ kg	enz	ahdx	li	21	gavage	88914
mouse	Chlorpyrifos	10	25	mg/kg/d	mph	wght	fet	12	gavage	93131
mouse	Malathion	0.1	10	mg/kg	enz/bcm	genz/H2O2	prt	0.167	gavage	90632
rat	Chlorpyrifos	1	5	mg/kg	cel	rsbc	br	365.28	DT	80972
rat	Chlorpyrifos	0,1	1	mg/kg	enz	cest	pl	91	FD	52006
dog	Chlorpyrifos	0.01	0.03	mg/kg	enz	cest	pl	7	DT	37866
rabbit	Chlorpyrifos	18.2	28.32	mg/kg	phy	htrt	-	90	DR	92599
mouse	Bentazon	400	600	mg/kg	mph	wght	li	Liver	10	90435

DISTRIBUTIONS AND EXTRAPOLATION EQUATIONS USED TO CONSTRUCT THE SSD IN @RISK

Table A. *The calculation path to construct the distributions of the different species for the SSD, these are the equations and distribution used in @risk*

Species B	start value (mg/day/kg) C	distribution of the value after extrapolation between LOAEL and NOAEL D	distribution of the value after extrapolation between substances E	time correcting factor F	simulation distributions G
Mouse	1.72	1.72	1.72	1	**RiskOutput(;"slutvärde";1) + E4*F4**
Rat	0.37	0.37	0.37	1	**RiskOutput(;"slutvärde";2) + E5*F5**
Hamster	100	100	100	1/39	**RiskOutput(;"slutvärde"; 3) + E6*F6**
Cat	10	10^RiskNormal((LOG(C7)*1,2074-0,9853); 0,516; RiskTruncate(0;))	10^RiskNormal((LOG(D7)*1,2074-0,9853); 0,516; RiskTruncate(0;))	1/39	**RiskOutput(;"slutvärde";4) + E7*F7**
Dog	0.018	0.018	0.018	1	**RiskOutput(;"slutvärde";5) + E8*F8**
goat	75	75	10^RiskLognorm(((LOG(D9)*1,0269)+0,6263); 0,32078; RiskTruncate(0;))	RiskUniform(0,001;0,01)	**RiskOutput(;"slutvärde";6) + E9*F9**
cattle	300	RiskNormal((LOG(C10)*1,2074-0,9853); 0,516; RiskTruncate(0;))	10^RiskNormal((D10*1,0269+0,6263); 0,32078; RiskTruncate(0;))	RiskUniform(0,001;0,01)	**RiskOutput(;"slutvärde";7) + E10*F10**
Sheep*	12.5	10^RiskNormal((LOG(D11)*1,2074-0,9853);0,516; RiskTruncate(0;))	RiskNormal(C11*0,6997+1,428; 2,239; RiskTruncate(0;))	1/7	**RiskOutput(;"slutvärde";8) + E11*F11**
rabbit	18.72	18.72	RiskNormal(D12*0,6997+1,428; 2,239; RiskTruncate(0;))	1/7	**RiskOutput(;"slutvärde";9) + E12*F12**

* The calculations were reversed (see Chapter 7.4.1.2), i.e. the extrapolation between substances was calculated first.