# PESTICIDE LEVELS AND ABSORBED DOSES INSIDE IOWA HOMES OVER TIME
# FARM FAMILIES' POTENTIAL LONG-TERM EXPOSURES

by

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To my wife Rebekah, parents Silvister and Rajamani, and sister Suneetha for their never ending support, faith, and encouragement to help me achieve my dissertation goals

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## ABSTRACT

Background: The hazards of chronic low-level pesticide exposures inside homes, have received relatively less attention. Few studies have found that farm homes have a greater frequency of detectable pesticide concentrations and higher levels in dust and farmer's urine; however research thus far does not provide answers concerning the long-term potential bioavailability of pesticides inside homes and its risk factors. The purpose of this study was to investigate pesticide levels in Iowa homes during one year and assess the relationship between exposure levels and potential sources of pesticide contamination.

Methods: The study involved farming-based sampling surveys of the target pesticide atrazine among 32 farm families and their homes in a three-county area of Iowa. The families were surveyed during the planting season (April-June) and non-planting season (November-December). Dust samples were collected from four locations within the homes and analyzed for atrazine. Two spot-urine samples were collected from spouses and children and analyzed for atrazine and six of its metabolites. Information was gathered through questionnaires to evaluate factors affecting pesticide migration inside homes.

Results: The first study found that dust in farmers' homes was contaminated with atrazine during both seasons and these concentrations significantly decreased over a period of one year. In the second study, urine samples of all study subjects had detectable atrazine and/or its metabolites in both seasons. Mean total urinary atrazine concentrations did not significantly decrease over a period of one year. The third study found that dust concentrations of some of the four locations in the study homes were positively

correlated with the total urinary atrazine levels of study subjects. A considerable number of atrazine dose estimates of farmers and their spouses were above the EPA chronic population-adjusted reference value.

Conclusions: This study found that all homes located on a farm had atrazine contamination inside them. The whole family average total urinary atrazine concentrations did not decrease over one year. The positive association of dust atrazine concentrations with total urinary atrazine concentrations indicates that the absorbed doses of atrazine are associated with the presence of atrazine in their homes.

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# CHAPTER 1

## INTRODUCTION

"The secret of National Health lies in the homes of the people". This statement by Florence Nightingale emphasizes the role of the home environment in the life and health of individuals. Families are potentially exposed to contaminants that are brought into the home or through substances used in the home. Residential environments geographically located close to farms may be susceptible to exposures as a result of farm operations. In particular, agrichemicals such as pesticides may be transported inside people's homes via air or work or personal activities that result in residues being brought into the home on clothing or skin. The pesticides may then enter the bodies of residents through inhalation, ingestion, and dermal contact.

In farm homes, families may be exposed to pesticides through home contamination even though they may not participate in farming activities involving pesticide use (Eskenazi et al., 1999). This can occur by environmental migration of pesticides from farm fields to homes (Fig 1.1). Residential environments in close proximity to farm operations where pesticides are used may be contaminated through a variety of routes--airborne spread, tracking of contaminated soil on shoes into the home, deposition on clothing, and deposition on pet animals. Indirect inhalation and dermal exposure may occur through redistribution of pesticides via air to surfaces (Quackenboss et al., 2000).

In addition, families are exposed to pesticides on food, and in homes that have been sprayed with pesticides. Indirect inhalation and dermal exposure of farm families to pesticides may occur through redistribution of pesticides from home surfaces by

volatilization and condensation and resuspension and settling. A recent study by Lewis et al. (2001) documents rapid translocation of diazinon and chlorpyrifos within the home following indoor and outdoor applications. Contamination of the indoor farm home environment with pesticides affects all family members, including children.

**Figure 1.1: Application of the Herbicide Atrazine using Groundboom Operation in Johnson County, Iowa, May 2005**

Concern for pesticide exposure among the children of farmers and farm workers was raised by the National Institute for Occupational Safety and Health (NIOSH) with the Report to Congress on Workers' Home Contamination Study conducted under the Workers' Family Protection Act (29 U.S.C. 671a) (NIOSH, 1995). Children are at an increased risk for exposure to pesticides because of their susceptibility to receive higher doses due to smaller body size and weight compared to adults and their activities that

bring them in contact with home surfaces. Also, their developing body organs are more susceptible to the effects of exposure, creating greater opportunities for the long-term effects of pesticide exposure to become manifest.

A few studies have found that farm homes have a greater frequency of detectable concentrations of pesticides and higher levels in dust than in reference homes, potentially leading to greater exposure to pesticides among family members (Camann et al., 1997; Bradman et al., 1997; Simcox et al., 1995). Most of this work has focused on organophosphorus pesticides (OPs). Pesticide urine concentrations among the children of farmers and farm workers have been shown to be elevated when compared to children of non-farm families (Loewenherz et al., 1997). A study was conducted by Sanderson et al. to measure alachlor metabolites in the urine of commercial pesticide applicators who were applying this pesticide to corn and soybean crops. This study found that alachlor's urine metabolite concentrations measured by enzyme linked immunosorbent assay (ELISA) were consistently higher than the respective high performance liquid chromatography (HPLC) measurements, nevertheless a high correlation was observed between the ELISA and HPLC measurements (Sanderson et al., 1995). Another study conducted to monitor pesticide exposures of children of agricultural workers revealed that these applicator children experienced higher exposure to OPs than did reference children in the same community (Loewenherz et al., 1997). Lewis et al. (2001) found that chlorpyrifos residues in indoor air and carpet were higher within a few days after an exterior residential application, than before the application, and suggested that track-in is the principal source of these residues. The United States Environmental Protection Agency (EPA) has attempted to address the issue of children's exposure in farm homes in

their standard operating procedures for residential exposure assessment. However, the EPA acknowledges that the research to date, although providing useful insight, does not provide answers regarding the potential bioavailability of pesticides from this source of exposure. According to Curwin et al. (2005a, 2005b, 2005c), there is a need for new pesticide exposure methods. Some of the recommendations are to achieve greater sophistication in exposure studies including biomonitoring and validation studies.

A study of agricultural health in Iowa is being supported by the Great Plains Center for Agricultural Health. The Keokuk County Iowa Rural Health Study (Stromquist et al., 1997) is a population-based, prospective health study of an agricultural community in Iowa. Evaluations of respiratory disease, injury, and other health outcomes in relation to environmental and occupational exposures are being investigated. In collaboration with this study, NIOSH conducted a study of in-home pesticide levels in 25 farm and 25 non-farm homes (Curwin et al., 2005b). Air, surface wipe, and dust samples were collected from these homes and analyzed for the presence of several pesticides: acetochlor, alachlor, chlorpyrifos, 2, 4-D, atrazine, metolachlor, and glyphosate. It was noted that chlorpyrifos, glyphosate, and 2, 4-D were found in air, surface wipe, and dust samples in both farm and non-farm homes. Higher levels of atrazine and metolachlor found in the samples collected from different locations in the homes suggested that these pesticides were potentially brought into the home on the farmer's shoes and clothing. Also, it was found that acetochlor was sprayed by a few farmers and alachlor was not sprayed at all. In part this was reflective of the small sample size in this study.

This study also concluded that some of the household characteristics such as age of the carpet, frequency of carpet vacuuming, presence of doormats, presence of pets, and age of the homes were not associated with pesticide levels in dust. The pesticide levels in this study were evaluated for any association with distance of the fields from the homes and found that the distance to pesticide treated fields had no correlation with the pesticide levels found in dust of non-farm homes. The distance to the fields was not analyzed for the farm homes since they were all located less than quarter of a mile from the treated fields. This study found that dust was a more useful sample media than wipe and air samples for the evaluation of pesticides at low levels in study homes. This study concluded that farm homes have higher concentrations of pesticides than non-farm homes. Further, it was found that farms that spray atrazine and metolachlor have higher concentrations of those pesticides inside their homes than other homes where residents did not spray those pesticides.

Although very informative, the Curwin et al. (2005a, 2005b) study was not able to clearly determine risk factors affecting pesticide levels and absorbed doses and was only conducted during the planting season. However, its results laid the foundation for this dissertation's goal to collect additional data to more fully learn how families are exposed to pesticides through in-home migration and to measure concentrations several months after the application season had ended. This dissertation will evaluate the risk factors associated with pesticide levels in farm homes and examine the persistence of pesticides in homes long after the planting season.

**Literature Review**

According to the EPA, in the year 2001 approximately 1200 million pounds of pesticides were applied in the United States, out of which 550 million pounds (46%) were herbicides. Farmers are the largest group of registered pesticide applicators according to the Agricultural Health Study (Alavanja et al., 1996). The potential for exposure of children to pesticides is a serious concern (Fenske et al., 2000a, 200b). Children are potentially exposed to pesticides that are brought into the home or used in the home. In farm homes, families, particularly children, may be exposed to pesticides through home contamination even though they may not participate in farming activities involving pesticide use (Eskenazi et al., 1999). Differences in children's physiology, behavior patterns, and hygiene may result in significantly greater exposures to environmental contaminants than adults due to intimate contact with pesticide residues on carpets or uncovered floors when playing inside and yard dirt when playing outside (Davis et al., 1990; Calabrese et al., 1991). It has been estimated that children under the age of 5 ingest 2.5 times more soil from around the home than adults yet possess only 20% of the body weight (Binder et al., 1986; Calabrese et al., 1989). Children, especially younger children, may also be more susceptible than adults to the toxic effects of pesticides, due to the sensitivity of developing organ systems. Federal pesticide regulators in Canada and the U.S. consider dietary but not residential and environmental exposure to children when evaluating the safety of agricultural pesticides.

Concern for pesticide exposure among the children of farmers and farm workers was raised by the National Institute for Occupational Safety and Health (NIOSH) with the Report to Congress on Workers' Home Contamination Study conducted under the

Workers' Family Protection Act (29 U.S.C. 671a) (NIOSH, 1995). The Natural Resources Defense Council (NRDC) considers pesticides to be one of the top five environmental threats to children's health and considers farm children to be the most highly pesticide-exposed subgroup in the U.S. (NRDC, 1998).

Pesticide urine concentrations among the children of farmers and farm workers have been shown to be elevated when compared to children of non-farm families (Loewenherz et al., 1997). However, most of this work has focused on organophosphorus pesticides (OP's) and information on other pesticides which are more widely used such as crop herbicides is needed. The EPA has attempted to address the issue of children's and other family member's pesticide exposure in farm homes in their Standard Operating Procedures for Residential Exposure Assessment (EPA, 1997). The EPA acknowledges however, that the research to date, although providing useful insight, does not provide answers regarding the potential bioavailability of pesticides from this source of exposure (EPA, 1999).

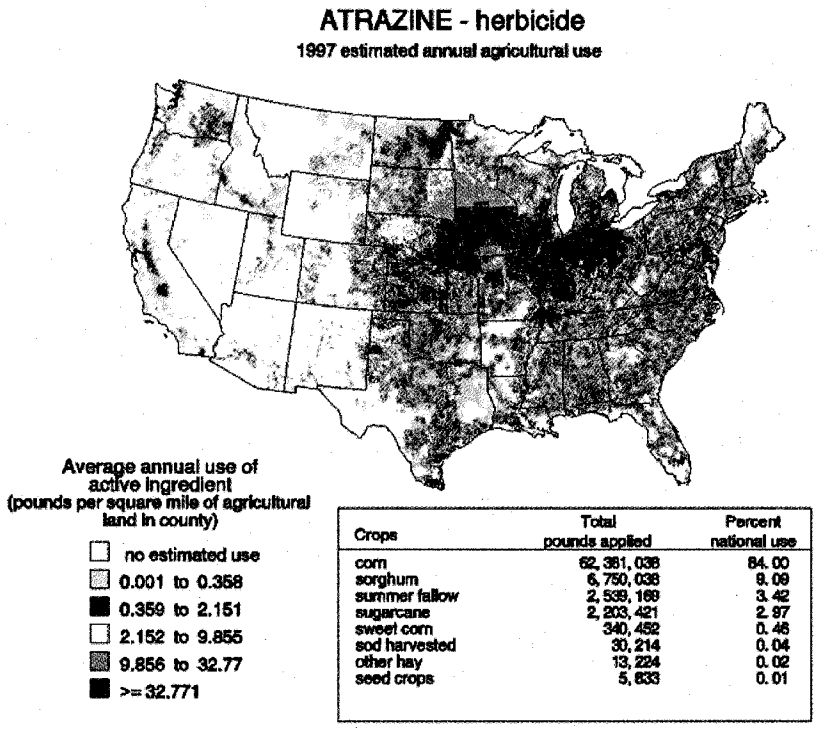
A study of children's exposure to pesticides in an area in Washington State with abundant orchard agriculture found that one sixth of the children had detectable levels of OPs on their hands versus none in the reference population. Also, 35 % of agricultural children exceeded the EPA's acute reference dose for azinphos-methyl of 3 μg/kg bw/day (Fenske et al., 2000a). A study of the distribution of 2,4-D in the air and on surfaces inside residences after lawn applications in Columbus, Ohio in which exposure estimates from various media for young children were compared, found that after lawn application, 2,4-D was detected in indoor air and on all surfaces throughout all homes. Resuspension of floor dust was the major source of 2,4-D in indoor air, with highest levels of 2,4-D

found in the particle size range of 2.5–10 μm. These levels were estimated to be about 10 times higher than the preapplication exposures (Nishioka et al., 2001). Another study of pesticide contamination in rural children's homes in California found that 10 of 33 pesticides analyzed were detected in house dust. Concentrations of diazinon and chlorpyrifos were higher in farm worker homes than non-farm worker homes, suggesting that children's exposure to diazinon could exceed the EPA's chronic reference dose (Bradman et al., 1997). A study by Curl et al. (2002) found that the take-home exposure pathway contributes to residential pesticide contamination in agricultural homes where young children are present.

**Pesticide Selection**

Atrazine(6-chloro *N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine), a triazine herbicide, used for controlling weeds in corn, sorghum, sugarcane and wheat fields came into wide usage in the 1970s (Fig. 1.2). This product was first introduced in 1958, and it has increased steadily over the past 44 years. It was applied to 65% of the planted corn acres in Iowa in 1999. In 2002 approximately 76 million pounds of Atrazine were applied in the United States (EPA's Overview).

**Figure 1.2: Estimated Annual Agricultural Use of Atrazine in 1997 (United States Geological Survey)**




| Crops | Total pounds applied | Percent national use |
|---|---|---|
| corn | 62,361,038 | 84.00 |
| sorghum | 6,750,038 | 9.09 |
| summer fallow | 2,539,169 | 3.42 |
| sugarcane | 2,203,421 | 2.97 |
| sweet corn | 340,452 | 0.46 |
| sod harvested | 30,214 | 0.04 |
| other hay | 13,224 | 0.02 |
| seed crops | 5,833 | 0.01 |

Atrazine was selected as the target pesticide for sampling and analysis because it is among the most commonly used pesticides in Iowa (USDA, 2000; Reynolds et al., 1998; Curwin et al., 2002b). This pesticide was previously studied by Curwin et al. in 2001 and there are sampling and analysis methods for atrazine in dust and atrazine metabolites in urine (Curwin et al., 2005b). It is important to make this study comparable to previous work so that comparison can be made between the studies and between results in planting and non-planting seasons. The herbicide atrazine was applied to 65% of the planted corn acres in Iowa in 1999. The dust samples were analyzed for the target pesticide. Studies that have conducted multiple pesticide residue analysis in homes have found detectable residues of this pesticide not associated with a particular spraying event

(Camann et al., 1997; Bradman et al., 1997; Colt et al., 1998; Lewis et al., 2001) indicating it is widely found in rural homes. Many other pesticides are not as widely used, do not have good environmental sampling and analysis methods, and if they are sampled for, often result in non-detectable concentrations. Therefore, atrazine will serve as a good marker of potential pesticide contamination of homes and allow for evaluation of how pesticides may migrate into homes.

**Product Identity and Uses**

Most of the available atrazine products are 95% pure, almost 3% above the ideal state. This quantity of application is a point of concern in terms of environmental protection and health preservation, as expanded use results in a ubiquitous distribution of atrazine in the environment. Some of the trade names/synonyms for this chemical are as follows: Aatrex; Actinite PK; Akticon; Argezin; Atazinax; Atranex; Atrataf; Atred; Candex; Cekuzina-T; Chromozin; Crisatrina; Cyazin; Fenamin; Fenatrol; Gesaprim; Griffex; Hungazin; Inakor; Pitezin; Primatol; Radazin; Strazine; Vectal; Weedex A; Wonuk; Zeapos; Zeazine (U.S. E.P.A Technical Fact Sheet on Atrazine).

**Physical and Chemical Properties**

Atrazine is a colorless crystalline powder with a low vapor pressure. It has a melting point of 175-177 degrees Celsius. It is readily soluble in many organic solvents such as methanol, diethyl ether, dimethyl sulfoxide, chloroform, etc. It is slightly soluble in water, yet compared to other synthetic organic compounds it is quite soluble. Atrazine is considered to be stable in the dry state, but is hydrolyzed in acid or alkaline solutions

(World Health Organization, 1990). Two primary characteristics of atrazine that increase risks of contamination of community water supplies are its mobility and resistance to metabolism (Kettles et al., 1997).

**Atrazine Fate and Transport in the Environment**

After the application of atrazine on farm fields, atrazine enters into the environment, particularly in soil and water. Its primary degradation can occur via soil bacteria and abiotic processes with an environmental half-life of a few weeks to several months (ATSDR and Mandelbaum et al., 1993). Atrazine is transported to water bodies by run-off from fields, in rainfall, due to misapplication, and improper disposal. This event is of great concern when those water bodies happen to be "source water" for drinking water supplies, as source water contamination may increase the risk of exposure through consumption. The environmental fate of atrazine is very complex. Atrazine is considered to be mobile and is transported throughout the environment due to its solubility in water. It is transported to surface water via run-off, spray drift and atmospheric transport. Atrazine has a history of being detected in rainfall, groundwater, and surface waters. These characteristics make atrazine a potential contaminant of all types of freshwater bodies. The resistance of atrazine to abiotic hydrolysis and direct aqueous photolysis and its moderate susceptibility to degradation in soil supports the fact that atrazine doesn't undergo rapid degradation on foliage. Atrazine has been observed to be more persistent in colder climate (World Health Organization, 1990). However, limited data exists as to the soil conditions and watershed processes that may enhance transport.

**Atrazine Exposure, Metabolism, and Excretion**

Atrazine is easily absorbed from the gastrointestinal tract but only to a limited extent through the skin (World Health Organization, 1990). Studies on rats showed that this herbicide and its metabolites bind effectively to red blood cells and to tissues of some of the major organs. Atrazine is rapidly eliminated from the body through urine and feces. Cardiac toxicity was observed in dogs after long-term oral administration of atrazine. Some of the effects observed in rats and mice, after experimental studies, were reduced food intake, decreased weight gain, and toxic effects, such as muscle and retinal degeneration, necrosis of the liver, and hematological effects. In addition, an increase in mammary tumors was observed in rats (World Health Organization, 1990).

The potential of atrazine to cause carcinogenesis in humans is still under research. It is known that xenoestrogens promote cancer by enhancing the production of genotoxic estrogens and mutations in cells. Exposure to excess estrogen is considered to be a risk factor for the development of breast cancer (Kettles et al., 1997). The National Institute of Environmental Health Sciences has been conducting significant research for determining the role of environmental estrogens and toxicological substances in initiating and/or promoting breast cancer. The International Agency for Research on Cancer (IARC) and the EPA has classified atrazine as a "possible" human carcinogen, as it was placed in category "C" under the cancer assessment guidelines (EPA's Overview). Atrazine has a potential to cause many acute and chronic health effects. Some of the acute health effects include muscle spasms, congestion of heart, lungs and kidneys, hypotension, antidiuresis, adrenal degeneration, etc (EPA Technical Fact Sheet on Atrazine). Documented chronic health effects in humans due to the consumption of

atrazine contaminated water are weight loss, mammary tumors, muscle degeneration, cardiovascular damage, and retinal degeneration.

**Conclusion**

This dissertation is aimed at evaluating the risk factors associated with pesticide levels in farm homes and examines the persistence of pesticides in homes long after the planting season. Additionally, this dissertation aims to study the relationship between family member's pesticide exposure, home pesticide contamination and factors influencing pesticide contamination.

There are three research objectives in this dissertation:

1) provide an analysis and evaluation of the concentrations of atrazine in vacuum dust samples from farm family homes in Iowa and assess the association between various factors, such as amount of atrazine used and family hygienic practices with atrazine levels in the dust, and to study the changes in pesticide concentrations in farm homes over time;

2) provide an analysis and evaluation of the concentrations of atrazine metabolites in urine samples from farm family members in Iowa and assess the association between various exposure factors and the urine metabolite levels, and to study the changes in urine pesticide concentrations in farm families over time; and

3) provide an analysis and evaluation of the correlation between the atrazine levels in the dust samples and the atrazine metabolite levels in the urine samples, study the changes in these associations over time, and study the comparisons of these results with toxicological data and assessment of potential farm family health risks.

These studies are discussed in chapters 2, 3, and 4. Chapter 5 discusses the findings of each study, their strengths and limitations, and how they can be used in the development and testing of interventions that can reduce pesticide contamination and potential exposures inside farm homes.

# CHAPTER 2

# VACUUM DUST CONCENTRATIONS AND VARIATION OF PESTICIDE CONTAMINATION IN FARM HOMES BETWEEN PLANTING AND NON-PLANTING SEASONS IN IOWA

## Introduction

Agricultural chemicals used on farms include a wide variety of pesticides such as herbicides, insecticides, fungicides, and rodenticides. The annual pesticide application in the United States is approximately 1200 million pounds (EPA. Pest. Indust. Market Estimates, 2001). Of all these, the most used are herbicides: approximately 46% of all the pesticides (EPA. Pest. Indust. Market Estimates, 2001). In 1999 this constituted the usage of approximately 550 million pounds of herbicides in the United States. Farmers are at risk of direct exposure to these pesticides during their handling, mixing, loading, and application of pesticides and farm pesticide equipment maintenance activities. It has been shown that pesticides sprayed outside a home can be transported into the home within a day after spraying (Lewis et al., 2001).

Study of the risk of adverse health effects from pesticide exposures requires an understanding of the specific agricultural chemicals used, routes of exposure, toxicity, duration of exposure, and the absorbed doses. The specific work characteristics of the individuals involved in these agricultural practices, and their use of protective equipment are key to assessing pesticide exposures and the potential for adverse health effects. In spite of significant research being carried out to characterize exposures in different agricultural settings, research data that can provide necessary information for risk estimation and characterization are not adequate (Reynolds et al., 1998). Residues from

recently sprayed pesticides can show up in dust, however, dust can also act as a reservoir for pesticides used on a long-term basis and even for pesticides that are no longer used but are environmentally persistent (Bradman et al., 1997; Colt et al., 1998; Lewis et al., 1994; Lewis et al., 2001).

In 2001, NIOSH conducted a study of in-home pesticide levels in 25 farm and 25 non-farm homes (Curwin et al., 2005b). Air, surface wipe, and vacuum dust samples were collected from these homes and analyzed for the presence of several pesticides: acetochlor, alachlor, chlorpyrifos, 2,4-D, atrazine, metolachlor, and glyphosate. It was noted that chlorpyrifos, glyphosate, and 2,4-D were found in air, surface wipe, and dust samples in both farm and non-farm homes. Higher levels of atrazine and metolachlor found in the samples collected from locations in the homes where applicators entered or changed clothes suggested that these pesticides were brought into the home on the farmer's shoes and clothing. Also, it was found that acetochlor was sprayed by a few farmers and alachlor was not sprayed at all, therefore these pesticides were not detected frequently in this study. Curwin found that pesticide concentrations were detected on a few of the wipe samples and that vacuum dust samples are the best matrix for indicating the extent of pesticide contamination inside homes (Curwin et al., 2002a). This result is also supported by work conducted in Minnesota (Lioy et al., 2000).

Curwin's study also concluded that some of the household characteristics such as age of the carpet, frequency of carpet vacuuming, presence of doormats, presence of pets, and age of the homes were not associated with pesticide levels in dust. The pesticide levels in this study were evaluated for association with distance of pesticide-treated fields from the non-farm homes and were found to have no correlation. The distance to the

fields was not analyzed for the farm homes since they were all located less than quarter of a mile from their treated fields. This study concluded that farm homes have higher concentrations of pesticides than non-farm homes. Further, it was concluded that farmers who sprayed a particular pesticide outside their homes have higher concentrations of that pesticide inside their homes than other homes where the occupants did not spray that pesticide. This finding was more specific to atrazine and metolachlor than the other pesticides under study, but those were the most commonly used of the seven pesticides.

Although very informative, this study was not able to clearly determine specific risk factors affecting pesticide levels and absorbed doses and was only conducted during the planting season. However, the results of the study laid the foundation for the current study in collecting additional data to more fully learn how families are exposed to pesticides through in-home migration and to measure concentrations several months after the application season had ended. The objective of the current study is to provide an analysis and evaluation of the concentrations of atrazine in vacuum dust samples from farm family homes in Iowa and assess the association between various factors, such as amount of atrazine used and family hygienic practices, and atrazine levels in the dust, and to study the changes in pesticide concentrations in farm homes over time.

## Methods

### Study Design

This study involved sampling surveys of 32 farm families and their homes which used the target pesticide atrazine. Ten of the farm families had children. Environmental pesticide levels were assessed by collecting dust samples from four locations within the

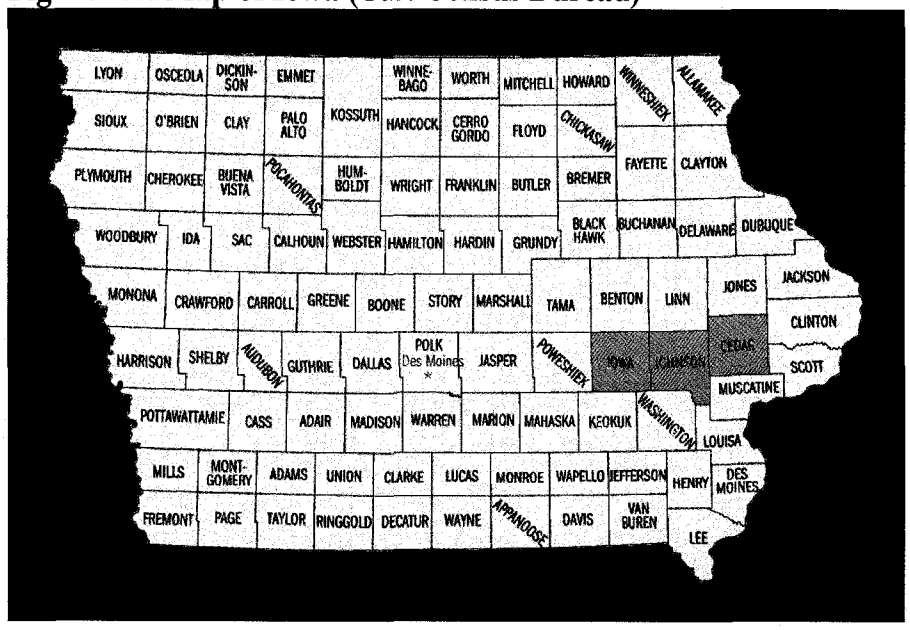
homes: entryway, living room, master bedroom, and kitchen. The samples were only analyzed for the herbicide atrazine. In addition, information was gathered through questionnaires and by observation to document information about the home, pesticide usage in and around the home, application techniques, work practices, use of personal protective equipment (PPE), and procedures for handling and cleaning work clothing. The questionnaire was used to evaluate factors affecting pesticide migration inside the home. These samples determined the location of pesticides and potential exposure pathways.

The study focused on measuring atrazine for vacuum dust collected from home floors. There were limitations to the study design. Not all exposure pathways were sampled. Food was potentially a source of pesticide exposure in farm families, but beyond asking questions about participants' gardens, no data were collected on food pesticide residues. Similarly, dermal exposure could be a significant route of exposure, but no effort was made to quantify dermal deposition.

Study Population and Recruitment

Subjects were recruited by contacting families living on a farm and designated in the Iowa Agricultural Census as farming at least 100 acres of tillable cropland in Cedar, Iowa, or Johnson counties (Fig. 2.1).

**Figure 2.1: Map of Iowa (U.S. Census Bureau)**




The farmers' information was acquired through the county plat books of the three counties under study. A total of 242 homes were randomly selected and sent an initial letter describing the study and requesting their participation. Individuals who refused to participate returned a "do not call statement" that was included along with the initial letter; 130 (54%) of the 242 homes that were sent the initial letter declined to participate in this study. Those individuals from whom this statement was not received were called within a period of two weeks of sending the initial contact letter; 32 of the remaining 112 homes met the requirement of planning to apply atrazine during the planting season and agreed to participate in the study. Ten of the 32 homes recruited for this study happened to be farm homes that farmer-applied their pesticides and the remaining 22 homes hired a commercial applicator for pesticide application in their farm fields. The average age of the farmers in this study was 60 years and 34% of the farmers did their own pesticide mixing, loading, and spraying, whereas 66% of the farmers hired a commercial applicator

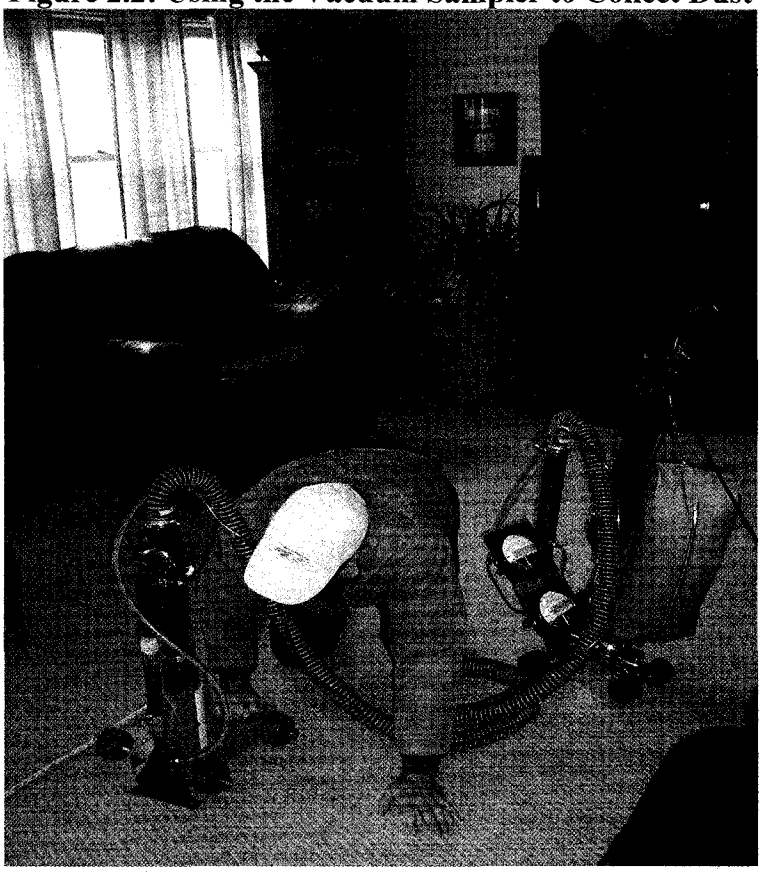
for these operations. Groundboom application of pesticides was used by all the farm homes in this study. If a farm family who planned to apply atrazine during the planting season declined to participate in this study, they were replaced by another randomly selected family until between 30 to 40 (the number of homes which could logistically be sampled) farm families expected to apply atrazine were recruited. During the first visit the investigator explained the study, answered questions, and obtained informed consent to participate. Each house was visited twice—once during the planting season (April-June) and once approximately six months after the planting season (non-planting season, November-December) of 2005. Farmers were given a telephone number to call study personnel to inform them that pesticide spraying had begun and the first visit was scheduled within a few days after atrazine was applied to cropland. The second visit was basically a repeat of the first visit except the questionnaire was modified to collect information about post-planting spraying of atrazine.

Sample Methods

Dust samples were collected from carpets using a high-volume surface sampler (HVS-3, Cascade Stamp Sampling Systems) following the American Society for Testing Material (ASTM) Standard Practice for Collection of Dust from Carpeted Floors for Chemical Analysis (ASTM D 5438-94, 2000). Briefly, the method involved sampling a measured area of approximately one square meter. The vacuum sampler, which contains a cyclone and a catch bottle, was passed over a strip of carpet in a straight line back and forth four times. This process was repeated with subsequent adjacent strips until the desired area had been sampled. A minimum of 0.5 grams of dust was desired, but care was taken to avoid filling up the cyclone catch bottle. If the desired amount of dust was

not collected, an adjacent 1 $m^2$ area was sampled. If not enough area existed to collect a sufficient quantity of dust for analysis, then dust samples from adjacent locations had to be combined to ensure a sufficient quantity of dust. A new catch bottle was used for each sample. Between samples, the vacuum and cyclone were cleaned with isopropanol to ensure that there was no contamination of samples from one home to another. Dust samples were typically collected from carpets in the entryway, living room, master bedroom, and kitchen (Fig. 2.2).

**Figure 2.2: Using the Vacuum Sampler to Collect Dust**



Analytical Method for Analysis of Atrazine in Dust

The dust samples were analyzed by Battelle Memorial Institute (Columbus, Ohio). According to the analysis report prepared by Marcia Nishioka and Charles Knott, "The dust samples were sieved to 150 μm using a 100 mesh stainless steel sieve. The fine dust was weighed to two significant figures after the decimal (e.g., 4.76 g) and returned to the original sample bottle. A 0.5 g aliquot of each fine dust sample was weighed out into a centrifuge tube and spiked with 250 ng of the surrogate recovery standard (SRS) $^{13}C_3$-atrazine. A 12 mL volume of 1:1 (v:v) hexane:acetone was added; the dust was sonicated for 10 min (Branson Sonicator 5210) for extraction and then centrifuged for 10 min at 3000 rpm (Forma Scientific) to settle the dust pellet. From the 12 mL extract, 10 mL was drawn off to a Kuderna-Danish (KD) tube and concentrated to 1 mL. An additional aliquot of 5 mL of hexane was added, and the extract was reconcentrated to 1 mL.

A 1000 mg silica SPE cartridge (BakerBond, JT Baker) was conditioned in sequence with 20% acetone in ethyl acetate, dichloromethane (DCM), 15% ethyl ether in hexane, and hexane. The sample extract was added to the cartridge with hexane rinses. The cartridge was eluted in sequence with 3 mL hexane, 2 x 6 mL of 15% ethyl ether in hexane, 6 mL of DCM, and 3 x 6 mL of 20% acetone in ethyl acetate. The first three eluants were discarded, and the acetone in ethyl acetate elutions was collected as one fraction. This eluant was concentrated to 1 mL using KD concentration. The internal standard (IS) for quantification, dibromobiphenyl, was added to the extract to give a final concentration of 100 ng/mL.

Each sample batch included 10 field samples, one field sample analyzed in duplicate, a field sample spiked with either 250 ng or 2500 ng of atrazine, and a solvent method blank fortified with only the SRS. The extracts and standards were analyzed using gas chromatography/mass spectrometry in the multiple ion detection mode (GC/MS/MID) with an HP 6890 GC interfaced to an HP 5973 MSD. The samples were analyzed in a run order with samples interspersed between standards. The internal standard method of quantification was used, with a linear regression calibration curve established for atrazine and the SRS. For samples where the atrazine exceeded the calibration range, the sample extract was diluted, respiked with IS, and reanalyzed. The recovery of the SRS is reported for each sample, as well as the SRS-corrected concentration of atrazine in the dust". The quality control for atrazine analysis in the dust samples is included in Appendix C.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

According to this analysis report, "The LOQ is defined operationally as the lowest concentration at which there is 10:1 S:N (signal-to-noise) ratio for the quantification ion. The LOD is the value at which there is a 3:1 S:N ratio for the quantification ion. These values for this atrazine analysis are 5ng/g for the LOQ in dust and 2.5 ng/g for LOD in dust. Atrazine was present in all samples at levels well above the LOD and LOQ. The concentrations of atrazine ranged from 3-593,111 ng/g in the field samples".

Data Analysis

Atrazine concentrations were reported in nanograms per gram of dust (ng/g) for the dust vacuum samples. The study examined the relationship between family member's pesticide exposure, home pesticide contamination, and factors influencing pesticide contamination. Descriptive summary statistics were used to describe the central tendency and variance of each variable measured. Analysis of variance (ANOVA) and multivariate analysis were used to determine if selected farm and personal practices influenced home contamination. The data collected from the interview were coded and analyzed to determine if any relationship exists between exposure and selected variables, and which of these variables affected home contamination and exposure the most. The variables that were considered included the number of acres of corn grown, the amount of atrazine applied, the age of the home, the age of the carpet, the number of hours of atrazine application, the number of days of atrazine application, how often the carpet was vacuum cleaned, whether the family owned a pet, application of any other pesticides inside the home, on the lawn or garden, change of clothes and shoes inside the home, laundering of clothes separately, and if the homes were located less than half a mile from the crop fields. The t-statistic was used to compare the difference in pesticide contamination and family exposure between farm homes that did their own spraying and those that hired a commercial applicator for spraying their fields. The vacuum dust samples were compared with each other for all four locations in the home for farmer-applied and commercial applicator-applied homes during both the planting and non-planting seasons to determine the best indicator of pesticide exposure and to see if any correlation existed between the samples. All statistical analyses were performed using

SAS system software, version 9.1 (SAS Institute, Inc., Cary, NC). All significance testing was performed at the 0.05 level of significance.

## Results

A total of 32 families were recruited where atrazine was applied to their farmland. This included ten farm families that had children 15 years old or less living in their household. A total of 73 subjects participated, of which 40 were male and 33 were female. Atrazine was applied to the corn fields by either the farmers (10 farm homes) or commercial applicators (22 farm homes).

The average number of acres sprayed, the duration of application, and the amount of atrazine used is displayed in Table 2.1. There were no significant differences in the number of acres sprayed, pounds of atrazine applied, or days applied between farm homes where atrazine was applied by farmers themselves and those where it was applied by commercial applicators. However, the farmers spent more hours applying atrazine than did the commercial applicators during the planting season for the targeted homes in this study.

**Table 2.1: Atrazine Application by Type of Pesticide Applicator**

| | Farmer Applicator (n = 10) | | Commercial Applicator (n = 22) | | p-value |
|---|---|---|---|---|---|
| | Mean (SD) | Range | Mean (SD) | Range | |
| **Avg. No. of Acres** | 381 (273.7) | 80 - 850 | 289 (233.2) | 50 – 900 | 0.33 |
| **Atrazine (lbs)** | 835 (599.5) | 175 - 1862 | 828 (669.3) | 144 – 2583 | 0.97 |
| **No. of days applied** | 5 (4) | 1 – 15 | 5 (12.2) | 1 – 60 | 0.95 |
| **No. of hours applied** | 7.3 (2.8) | 2 – 12 | 5 (3.3) | 1 – 14 | 0.05 |

*Student's t-test with p-value < 0.05 considered statistically significant

The average number of acres (mean = 216, sd = 123; obtained from the county plat books) farmed by the remaining 80 homes of the 112 homes that did not send the "do not call" statement back is significantly different from the average number of acres (mean = 318, sd = 246) farmed by the 32 study homes that were recruited (p-value = 0.03). The average age of the farmers in this study (mean = 60 years) is not significantly different than the farmers (mean = 65 years) of the remaining homes of the 112 homes that were not recruited into the study (p-value = 0.08). This age information was obtained through internet search of public information.

**Table 2.2: Atrazine Spraying Intervals for Planting and Non-Planting Seasons**

| | **Planting season (April-July)** | | | **Non-Planting season ( Nov-Dec)** | | |
|---|---|---|---|---|---|---|
| | Interval (days) | No. of homes | % of homes | Interval* (days) | No. of homes | % of homes |
| **Number of days between end of atrazine application and sample collection** | 0 | 4 | 12 | 188-190 | 7 | 22 |
| | 1-2 | 6 | 19 | 191-200 | 8 | 25 |
| | 3-4 | 6 | 19 | 201-210 | 2 | 6 |
| | 5 - 6 | 16 | 50 | 211-219 | 15 | 47 |
| | | | | | | |
| **Number of days atrazine applied to cropland before sample collection** | 1-2 | 11 | 35 | 1-2 | 12 | 38 |
| | 3-4 | 9 | 28 | 3-4 | 3 | 9 |
| | 5-6 | 3 | 9 | 5-6 | 0 | 0 |
| | >6 | 9 | 28 | >6 | 0 | 0 |

*Interval (days) in the non-planting season represents the number of days between end of atrazine application in the planting season and sample collection in the non-planting season.

The number of days between the end of atrazine application and sample collection and the total number of days atrazine was applied before sample collection are presented in Table 2.2 by planting and non-planting seasons. During planting season, samples were collected in half the homes within four days of the end of atrazine application and from 5 to 6 days after the end of application in the remaining half. For 35% of the homes

atrazine was applied for only one or two days before samples were collected and for 28% of the homes atrazine was applied for three or four days. During non-planting season, no samples were collected less than six months (183 days) after the end of atrazine application and all samples were collected before 220 days (7.2 months) after the end of application. Between the time of sample collection during planting season and the time of sample collection during the non-planting season, only 47% of the homes had additional days on which atrazine was applied—on 38% atrazine was applied for one or two days and on 9% atrazine was applied for three or four days.

Factors evaluated for association with atrazine levels in the dust are presented in Table 2.3. There were no significant differences for any of these factors between farmers whose sprayed their fields with atrazine compared with farmers whose fields were sprayed by commercial applicators (p-value = 0.95). Similarly, there is no statistical significance between farmer-applied and commercially-applied homes for the age of carpet in the farm homes (p-value = 0.30). Also, there were no significant differences between farmer-applied homes and commercially-applied homes for pesticide application inside the home, on the lawn, and in the garden within the past year. Likewise, there were no differences between farmer-applied homes and commercial applicator-applied homes for the presence of doormats, frequency of carpet vacuuming, presence of pets, and location of homes within half a mile of crop fields.

**Table 2.3: Household Characteristics of Farm Families by Type of Pesticide Applicator**

| Household Characteristics | Farmer Applicator | | Commercial Applicator | | p-value |
|---|---|---|---|---|---|
| | Mean (SD) | Range | Mean (SD) | Range | |
| Age of home | 61 (56.7) | 2 -166 | 60 (42.5) | 7 - 146 | 0.95 |
| Age of carpet | 37 (26.1) | 2 - 86 | 49 (33.4) | 7 - 106 | 0.30 |
| | | | | | |
| | No. of Homes | % Homes | No. of Homes | % Homes | p-value |
| Any pesticide applied inside home within past year | 5 | 16 | 8 | 25 | 0.46 |
| Lawns treated with any pesticide within past year | 4 | 13 | 10 | 31 | 0.77 |
| Garden treated with any pesticide within past year | 3 | 9 | 10 | 31 | 0.40 |
| Carpet vacuumed at least once a week | 8 | 25 | 20 | 63 | 0.38 |
| Presence of Doormats | 9 | 28 | 21 | 66 | 0.55 |
| Presence of Pets | 9 | 28 | 16 | 50 | 0.27 |
| Distance of the home from the farm < 0.5 miles | 10 | 31 | 21 | 66 | 0.49 |
| Launder clothes separate from rest of the family | 5 | 16 | 14 | 44 | 0.46 |
| Change work clothes inside the home | 7 | 22 | 14 | 44 | 0.72 |
| Change shoes inside the home | 7 | 22 | 11 | 34 | 0.29 |

*p-value < 0.05 is obtained from Chi-Square test is statistically significant

A total of 256 dust samples were obtained from the carpet, rugs, and floors of the farmers' homes during both the planting and non-planting season (Table 9). However, three of the four samples of one home for which a commercial applicator had applied atrazine in the planting season were lost during shipment to the laboratory for analysis, and samples (both planting and non-planting season's samples = 8) from that home were not included in any of further analysis. Therefore the total number of samples available for analysis was 248. The dust samples were primarily obtained from four different

locations in each home: the entryway, living room, master bedroom, and kitchen. Every effort was made to keep the four different locations constant over the 32 households. However, there was variation in location for a few homes. For example, some homes had an entryway that led directly into the living room. Some others had an entryway through the garage door. Some farmers were using the garage door entryway to get into the house, while some others used the front door to enter directly into the living room and others entered the home through the kitchen. The location that the farmer entered the house was recorded as the entryway and kept constant throughout the study. All atrazine dust concentrations are represented as geometric mean concentrations because the sample distributions were log-normally distributed.

During the planting season, the entryways and the kitchens had the greatest concentrations of atrazine in the dust samples. The living rooms (or rooms in which the family spent most of their time together) and the master bedrooms had similar mean atrazine concentrations. The concentration of atrazine in the household dust samples decreased by approximately an order of magnitude between the planting and non-planting seasons, but atrazine was still detectable in all the dust samples. Comparing the planting season atrazine concentrations with those of the non-planting season based on the concentration of atrazine in ng/g in Table 2.4, it is observed that the planting season atrazine concentrations are significantly higher than the non-planting season atrazine concentrations for all locations and for household averages (p-value $< 0.05$). The household average concentrations were calculated by averaging the mean atrazine concentrations of all the four locations in each study home. The geometric mean of the individual averages of the study homes was computed to obtain the household average

for all the four locations of all the homes. For both the planting and non-planting seasons, atrazine concentrations for the four locations inside the homes were not statistically significantly different from each other (p-value > 0.05).

**Table 2.4: Atrazine Concentrations in Dust Samples by Location within Homes and Season in Nanograms per Gram Dust (ng/g)**

| | Planting Season | | Non-Planting Season | |
|---|---|---|---|---|
| **Location** | **GM (GSD)** | **Range** | **GM(GSD)** | **Range** |
| **Entryway** | 687 (11.4)[a] | 5 - 139,200 | 47 (9.1)[b, c] | 1 – 1,896 |
| **Living Room** | 285 (15.3)[a, b] | 1 – 1,268,003 | 30 (7.3)[c] | 1 – 2,309 |
| **Master Bedroom** | 307 (9.2)[a, b] | 10 – 22,499 | 19 (7.1)[c] | 1 – 3,630 |
| **Kitchen** | 566 (10.6)[a] | 27 – 697,753 | 41 (11.8)[b, c] | 1 – 15,224 |
| **House Avg.** | 858 (11.02)[a] | 36.8 - 323230 | 49 (8)[b] | 2.8 – 5,550 |

[a, b, c] Geometric means with the same letter are not significantly different from each other based on p-value < 0.05 obtained from ANOVA.

In Table 2.5, the summary statistics of the atrazine concentrations for both planting and non-planting seasons are presented in nanograms of atrazine per square centimeter of floor sampled (ng/cm$^2$). Comparing the planting season atrazine concentrations with those of the non-planting season based on the concentration of atrazine in ng/cm$^2$, it is observed that the planting season atrazine concentrations are significantly higher than the non-planting season atrazine concentrations for the entryway and kitchen (p-value< 0.05). There was no statistical significance for the living room and master bedroom between the planting and non-planting seasons (p-value ≥ 0.05).

**Table 2.5: Atrazine Concentrations in Dust Samples by Location within the Homes and Season in Nanograms per Square Centimeter (ng/cm$^2$)**

| | Planting | | Non-Planting | |
|---|---|---|---|---|
| **Location** | **GM (GSD)** | **Range** | **GM (GSD)** | **Range** |
| **Entryway** | 0.39 (21)[a] | 0.001 - 87 | 0.015 (16.4)[b, c, d] | 4.6E-5 - 5 |
| **Living Room** | 0.03 (14.4)[a, b, c, d] | 4.4E-5 - 8 | 0.002 (11.3)[d] | 6.2E-6 - 0.4 |
| **Master Bedroom** | 0.04 (7)[a, b, c] | 0.001 - 3 | 0.003 (10.3)[c, d] | 6.1E-5 - 1 |
| **Kitchen** | 0.14 (17.7)[a, b] | 0.001 - 652 | 0.007 (37)[c, d] | 4E-6 - 62 |
| **House Avg.** | 0.31 (14.8)[a] | 0.009 - 164 | 0.017 (17.3)[b] | 0.0001 - 16 |

[a, b, c] Geometric means with the same letter are not significantly different from each other based on p-value < 0.05 obtained from ANOVA.

The geometric mean atrazine concentrations in household dust samples are compared for the four locations for both the planting and non-planting seasons by the type of pesticide application in Table 2.6. Atrazine concentrations in homes of farmers who applied their own pesticides for their entire acreage are compared with those of farmers who had their fields sprayed for pesticides entirely by a commercial applicator. The entryways and the kitchens remained the areas with the greatest concentrations regardless of whether atrazine was applied by the farmer or a commercial applicator, but this analysis shows that the homes of farmers who applied atrazine had greater concentrations than the homes where atrazine was commercially-applied. The data show the atrazine concentrations are on average about five or six times lower in homes for which a commercial applicator had applied atrazine. The data also show the atrazine concentrations in the non-planting season have on average decreased to less than 10% of the concentrations found during the planting season, for both homes where farmers applied and commercial applicators applied atrazine.

**Table 2.6: Geometric Mean Atrazine Concentrations in Dust Samples by Location within Homes, Season, and Type of Application in Nanograms per Gram Dust (ng/g)**

| | Planting | | Non-Planting | |
|---|---|---|---|---|
| **Location** | **Farmer (n = 10) GM (GSD)** | **Commercial Applicator (n = 21) GM (GSD)** | **Farmer (n =10) GM (GSD)** | **Commercial Applicator (n = 21) GM (GSD)** |
| **Entryway** | 1935 (18)[c] | 419 (8)[c, d] | 180 (9)[b] | 25 (7.1)[e] |
| **Living Room** | 460 (22)[a, c] | 227 (13.2)[a, d] | 74 (7.2)[a, b] | 19 (6.6)[b, e] |
| **Master Bedroom** | 1031 (8.1)[c] | 182 (8.2)[d] | 57 (5.6)[b] | 12 (6.7)[e] |
| **Kitchen** | 1901 (7.8)[c] | 309 (10.2)[d] | 179 (10)[b] | 21 (9.6)[e] |
| **House Avg.** | 2422 (9.8)[c] | 524 (10.6)[d] | 123 (156)[b] | 28 (6.4)[e] |

[a, b, c] Geometric means with the same letter are not significantly different from each other based on p-value < 0.05 obtained from paired t-test.

For the planting season, the average atrazine concentration for all the four locations of farmer-applied homes is significantly different than those of commercially-applied homes (p-value < 0.05). For the non-planting season, the average atrazine concentration for all the four locations of farmer-applied homes is significantly different than those of commercially-applied homes (p-value < 0.05). Comparing the planting and non-planting seasons, the average atrazine concentration of the entryway, master bedroom, and kitchen of farmer-applied homes in the planting season is significantly different than those of commercially-applied homes for the non-planting season (p-value < 0.05). The concentration of atrazine in the living room for the farmer-applied homes in the planting season is not significantly different than that of the commercially-applied homes in the non-planting season (p-value > 0.05). The atrazine concentrations of the

homes that had pesticides sprayed by commercial applicators were compared for both the planting and non-planting seasons. These results indicated that the entryway, living room, master bedroom, and kitchen mean atrazine concentrations were significantly different from those of the non-planting season (p-value < 0.05).

The household and farm characteristics were analyzed for associations with planting and non-planting average dust atrazine concentrations. PROC GLM was used to identify associations between continuous and categorical variables of the study homes as represented in Table 2.7. Among farmers who applied their own pesticides, the number of acres of corn fields, amount of atrazine applied, the number of days and hours of atrazine application were marginally positively associated with the mean atrazine concentration of farm homes in both the planting (GM =7.1 ng/g) and non-planting season (GM=5.04 ng/g). Among farmers who had a commercial applicator apply pesticides on their fields, the number of acres of corn fields, the amount of atrazine, and the number of days of atrazine application were positively associated with the planting season (GM =6.2 ng/g) and the number of acres of corn fields (p-value = 0.04), amount of atrazine applied (p-value = 0.04), and the number of days of atrazine application (p-value = 0.0008) were strongly associated with the mean atrazine concentration of farm homes in the non-planting season (GM=3.5 ng/g, p-value < 0.05). Also, the age of home and carpet were significantly associated with planting and non-planting season atrazine dust concentrations of commercially-applied homes (p-value < 0.05). Atrazine dust concentrations in these homes decreased with age of home and carpet in the planting and non-planting seasons.

**Table 2.7: Univariate Associations of Household and Farm Characteristics with Planting and Non-Planting Average Atrazine Dust Concentrations (ng/g)**

| | Homes with Farmer Applicator | | Homes with Commercial Applicator | |
|---|---|---|---|---|
| **Characteristic** | **Planting (p-value)** | **Non-Planting (p-value)** | **Planting (p-value)** | **Non-Planting (p-value)** |
| Age of home | 0.69 | 0.8 | 0.004* | 0.006* |
| Age of carpet | 0.56 | 0.45 | 0.01* | 0.03* |
| Number of corn acres sprayed with atrazine | 0.55 | 0.19 | 0.14 | 0.04* |
| Number of pounds of atrazine applied | 0.57 | 0.19 | 0.16 | 0.04* |
| Number of days of atrazine application | 0.15 | 0.47 | 0.003* | 0.0008* |
| Number of hours of atrazine application per day | 0.07 | 0.007* | 0.54 | 0.86 |
| Any pesticide applied inside home within past year | 0.65 | 0.34 | 0.15 | 0.33 |
| Lawns treated with any pesticide within past year | 0.65 | 0.70 | 0.13 | 0.1 |
| Garden treated with any pesticide within past year | 0.5 | 0.71 | 0.9 | 0.99 |
| Carpet vacuumed at least once a week | 0.43 | 0.35 | 0.32 | 0.19 |
| Presence of Doormats | 0.04* | 0.14 | 0.53 | 0.29 |
| Presence of Pets | 0.45 | 0.25 | 0.83 | 0.79 |
| Launder clothes separate from rest of the family | 0.03* | 0.006* | 0.96 | 0.44 |
| Change work clothes inside the home | 0.2 | 0.09 | 0.04* | 0.11 |
| Change shoes inside the home | 0.04* | 0.22 | 0.09 | 0.14 |

* p-value < 0.05 obtained from PROC GLM is statistically significant

Among farmers that applied their own pesticides, change of shoes outside the home (GM = 9.9ng/g) was significantly higher than those that changed their shoes inside the home (GM = 6.8 ng/g) when compared with the mean atrazine concentration (GM =

7.7ng/g, p-value = 0.04) during the planting season, and laundering their clothes separately from the rest of the family was significantly higher (GM = 9.2 ng/g) than laundering the clothes together with the family (GM = 6.3 ng/g) compared with the mean atrazine concentration in the planting season (GM = 7.7ng/g, p-value=0.03). Among farmers who had a commercial applicator for pesticide application, changing the farmer's clothes inside the home was significantly associated with the mean atrazine concentration in the planting season (GM = 6.2ng/g, p-value = 0.04). During the non-planting season, among farmers that applied their own pesticides, laundering their clothes separately from the rest of the family was significantly higher (GM = 6.6 ng/g) than laundering the clothes together with the family (GM = 3.4 ng/g) compared with the mean atrazine concentration in the non-planting season (GM = 5.04 ng/g, p-value = 0.006). The other variables were not statistically associated with the planting and non-planting mean atrazine concentrations.

Based on the above results, multivariate analysis was conducted using the step-wise selection method to study the associations of the planting and non-planting average dust atrazine concentrations with the significant continuous and categorical variables from the univariate analysis. The number of acres of corn applied with atrazine, the amount of atrazine applied, and the number of days and hours of atrazine application were found to be significantly associated with both planting and non-planting seasons' average dust concentrations (p-value < 0.05, $R^2 = 0.5$). Laundering clothes separately, changing shoes and work clothes outside the homes, and the presence of doormats were not significantly associated with both planting and non-planting average dust concentrations (p-value > 0.05).

For the planting season, the prediction equation is,

Planting Avg. Dust Conc. = 4.7 + 0.01 (corn acres) - 0.005 (amount applied) + 0.14 (days applied) + 0.25 (hours applied)

For the non-planting season, the prediction equation is,

Non-planting Avg. Dust Conc. = 1.9 + 0.01 (corn acres) - 0.005 (amount applied) + 0.11 (days applied) + 0.18 (hours applied)

**Discussion**

This study found that farmers' homes are contaminated with the herbicide atrazine during both the planting and non-planting seasons. Comparison of the home atrazine concentrations for the four locations based on the dust concentrations revealed that there was a significant difference between the concentrations in the planting and non-planting seasons. Mean atrazine vacuum dust concentrations based on the amount of dust collected (ng/g) significantly decreased over a period of one year. However, when the atrazine concentrations of homes in the planting season were compared to those in the non-planting season based on the area of dust collection ($ng/cm^2$) at each location in farm homes, it was found that the atrazine concentrations did not significantly decrease over a period of one year for all locations. The homes in this study were definitely contaminated by atrazine and are comparable to pesticide concentrations found in farm homes in the studies conducted by Curwin et al., (2005a), Simcox et al., (1995), and Bradman et al., (1997) also measured significantly higher pesticide concentrations in dust samples in farm homes compared to non-farm homes.

The farmers in this study differed in pesticide application. Some of the farmers applied their own pesticides to the crop fields, whereas the others hired a commercial applicator to apply the pesticides. This study found that both types of homes had significant concentrations of atrazine in the dust collected from the four locations in the planting and non-planting seasons. All the four sampling locations did not have a significant difference in atrazine concentrations between the homes that did their own spraying versus those that hired a commercial applicator, during the planting and non-planting seasons. Atrazine dust concentrations of homes that did their own atrazine application are significantly higher (except the living room) than those that had a commercial applicator apply atrazine. This finding is contrary to the common belief that farm homes that hire a commercial applicator to spray the pesticides on their fields will not be contaminated by pesticides applied on the fields. This study also revealed that the home average atrazine concentrations significantly decreased over a period of one year for farm homes that did their own atrazine application and those that hired a commercial applicator. This finding is novel as non-planting season pesticide concentrations have not been studied in the past and addresses one of the major limitations of Curwin's study. Even though atrazine dust concentrations decrease over time, it is important to note the presence of this pesticide in detectable quantities when no major pesticide application is taking place.

Several household and farm characteristics were studied for association with planting and non-planting dust atrazine concentrations and their associations compared to previous study (Curwin et al., 2005a, 2005b). Several of these variables were not associated and can be compared to similar findings in Curwin's study. The positive

associations between the planting and non-planting average atrazine concentrations and the number of acres sprayed with atrazine, the amount applied, and the number of days and hours spent spraying indicate that the larger the farm operation involving the use of atrazine, the greater the likelihood of atrazine being transported into the farm homes. The significant associations of the age of home and carpet of homes that hired a commercial applicator for pesticide application indicates the effect of time on the persistence of this pesticide in farm homes. The higher dust concentrations of atrazine among farm homes where farmers changed their shoes outside and laundered their clothes separately compared with those that changed their shoes inside and laundered their clothes along with the other family members can be explained by the huge amounts of atrazine applied on these crop fields that would significantly increase the likelihood of this pesticide being brought into the homes and being detected inside farm homes.

**Conclusion**

This study found that all homes located on a farm, whether they self-apply or commercially-apply pesticides had atrazine contamination inside the homes. The overall home atrazine concentrations for both the farmer-applied and commercially-applied homes were statistically significantly different between the planting and non-planting season. Comparison of atrazine concentrations using amount of dust collected and area sampled, found that the concentrations of atrazine were higher in the planting season compared to the non-planting season and decreased over a period of one year in these farm homes. Certain locations inside the farm homes had a difference in atrazine concentrations between homes where the pesticides were farmer-applied or commercially

applied. Atrazine concentrations significantly decreased over a period of one year in homes that farmer-applied and in homes that commercially applied. It is the amount of atrazine applied, the number of acres sprayed, and the time spent in atrazine application that determines the presence and persistence of this herbicide inside farm homes both in the planting and non-planting seasons.

# CHAPTER 3

# URINARY ATRAZINE CONCENTRATIONS AND ABSORBED DOSES FOR FARM FAMILIES' PESTICIDE CONCENTRATIONS OVER TIME

## Introduction

Agricultural chemicals used on farms include a wide variety of pesticides such as herbicides, insecticides, fungicides, and rodenticides. The annual pesticide application in the United States is approximately 1200 million pounds (EPA. Pest. Indust. Market Estimates, 2001). Of all these, the most used are herbicides: approximately 46% of all the pesticides (EPA. Pest. Indust. Market Estimates, 2001). In 1999 this constituted the usage of approximately 550 million pounds of herbicides in the United States. Farmers are at risk of direct exposure to these pesticides during their handling, mixing, loading, and application of pesticides and farm pesticide equipment maintenance activities.

Study of the risk of adverse health effects from pesticide exposures requires an understanding of the specific agricultural chemicals used, routes of exposure, toxicity, duration of exposure, and the absorbed doses. For example, adverse associations of prenatal organophosphate (OP) metabolites with mental development and pervasive developmental problems of children at 24 months of age was observed in a study done to investigate the relationship of prenatal and child OP urinary metabolite levels with children's neurodevelopment (Eskenazi et al., 2007). An assessment of atrazine and prostate cancer was studied by Alavanja et al. (2003) as part of a large prospective cohort study of pesticide applicators, known as the Agricultural Health Study (AHS), and reported on the risk of prostate cancer for individual pesticides within the cohort. Cancer

incidence among pesticide applicators due to atrazine exposure was also studied by Rusiecki et al. in the AHS.

Developing internally valid and generalizable farmworker exposure studies is a complex process involving many statistical and laboratory considerations (Barr et al., 2006a, 2006b, 2007, in press). Analytical methods developed and used for biological monitoring, and their use in pesticide exposure studies have been described by Barr et al. (2002). Atrazine that enters the mammalian systems typically undergoes biotransformation by the liver, of either the parent compound or the metabolites (Abel et al., 2004, Ademola et al., 1993; Hanioka et al., 1998; Hanioka et al., 1999; Lang et al., 1996; Lucas et al., 1993) and is excreted in urine. This excreted urine can contain less than 2% of unchanged atrazine (Catenacci et al., 1993; Meli et al., 1992). Human and rodent atrazine metabolites that were studied include diaminochloroatrazine (DACT), desethyl atrazine (DEA), desisopropyl atrazine (DIA)], hydroxy atrazine (ATZ-OH), hydroxy-DIA (DIA-OH), hydroxy-DEA (DEA-OH), diamino atrazine mercapturate (DAAM), DEA mercapturate (DEAM), DIA mercapturate (DIAM), and ATZ mercapturate (ATZM) (Ademola et al., 1993; Catenacci et al., 1990, 1993; Hanioka et al., 1998; Ikonen et al., 1988).

In order to accurately measure exposures to atrazine, it is necessary to measure multiple potential metabolites of this herbicide (Panuwet et al., 2007, in press). Most of the qualitative and quantitative analytical methods have been limited by the number of metabolites that could be detected, the selectivity of the analysis, and the sensitivity of the analysis. Therefore a novel, highly sensitive, on-line solid phase extraction-isotope dilution-high performance liquid chromatography-tandem mass spectrometry (On-line

SPE-HPLC-MS/MS) was developed by NCEH to quantify atrazine and its major metabolites in human urine samples (Panuwet et al., 2007 in press).

In 2001, NIOSH conducted a study of urinary pesticide levels among farmers and non-farmers in Iowa (Curwin et al., 2005a). Urine samples were collected from each participant on two occasions one month apart. The samples were collected were analyzed for the presence of several pesticides: acetochlor, alachlor, chlorpyrifos, 2,4-D, atrazine, and metolachlor. It was noted that for atrazine, acetochlor, metolachlor, and 2,4-D, farmers who reported applying the pesticide had significantly higher urinary metabolite levels than non-farmers, farmers who did not apply the pesticide, and farmers who had the pesticide commercially-applied (Curwin et al., 2005a). Among farmers who reported applying 2,4-D themselves, it was observed that some of the pesticide application variables such as time since application, amount of pesticide applied, the number of acres were marginally associated with 2,4-D urine levels. Also, time since application and farm size were marginally associated with atrazine mercapturate urine levels among those farmers that applied atrazine.

In the spring and summer of the same year, Curwin conducted a take-home pesticide study of children, mothers, and fathers living in farm and non-farm households in Iowa. The study subjects were sampled on two occasions one month apart for atrazine, metolachlor, glyphosate, and chlorpyrifos. This study revealed that urinary atrazine metabolite mean levels were significantly higher in children, mothers, and fathers from farm households compared to those from non-farm households (Curwin et al., 2007). It was also observed that urinary metabolites of chlorpyrifos were significantly higher in farm fathers and marginally higher in farm mothers, but metolachlor and glyphosate

levels were similar between the two groups. The geometric mean levels of the urinary metabolites for chlorpyrifos, metolachlor, and glyphosate were not significantly different between farm children and non-farm children. Also, farm children had significantly higher urinary atrazine and chlorpyrifos levels when these pesticides were applied by their fathers prior to sample collection than those of farm children when these pesticides were not recently applied (Curwin et al., 2007). These studies concluded that farmers who applied a particular pesticide had higher urinary metabolite levels of that pesticide. There was no significant difference in urine metabolite levels between farmers who had a pesticide commercially-applied to crops, did not apply the pesticide at all, or non-farmers.

Although very informative, this study was not able to clearly determine specific risk factors affecting pesticide levels and absorbed doses and was only conducted during the planting season. However, the results of the study laid the foundation for the current study in collecting additional data to more fully understand how families are exposed to pesticides and to measure urinary pesticide concentrations during the planting season and several months after the application season ends. The objective of the study is to provide an analysis and evaluation of the concentrations of atrazine metabolites in urine samples from farm family members in Iowa; to assess the association between various exposure factors and the metabolite levels, and to study the changes in pesticide concentrations in farm families over time.

## Methods

### Study Design

The study involved sampling surveys of 32 farm families and their homes which use the target pesticide atrazine. Ten of the farm families had children. Subjects were recruited by contacting families living on a farm and designated in the Iowa Agricultural Census as farming at least 100 acres of tillable cropland in Cedar, Iowa, or Johnson counties. The farmers' information was acquired through the county plat books of the three counties under study. A total of 242 homes were randomly selected and sent an initial letter describing the study and requesting their participation. Individuals who refused to participate returned a "do not call statement" that was included along with the initial letter; 130 (54%) of the 242 homes that were sent the initial letter declined to participate in this study. Those individuals from whom this statement was not received were called within a period of two weeks of sending the initial contact letter; 32 of the remaining 112 homes met the requirement of planning to apply atrazine during the planting season and agreed to participate in the study. If a farm family who planned to apply atrazine during the planting season declined to participate in this study, they were replaced by another randomly selected family until between 30 to 40 (the number of homes which could logistically be sampled) farm families expected to apply atrazine were recruited.

During the first visit the study was explained, the investigator explained the study, answered questions, and obtained informed consent to participate. Each house was visited twice—during the planting season and approximately six months after the planting season (non-planting season). Farmers were given a telephone number to call study

personnel to inform them that pesticide spraying had begun. The first visit was scheduled within a few days of atrazine application to cropland. The second visit was basically a repeat of the first visit except the questionnaire was modified to collect information about post-planting spraying.

Sample Methods and Analysis

During each home visit both spouses and children in the home were asked to provide two spot-urine samples, one in the evening of the day of the visit and one the following morning. The urine samples were collected in 500 ml nalgene bottles and the participants were asked to store the urine in their refrigerator or in a cooler with ice packs provided by the researchers. Two, 25 mL aliquots were removed, stored on dry ice, and shipped to the laboratory for analysis. The total volume of each urine void was recorded. One aliquot remained in storage in a -80 $^{o}$C freezer. The other 25-mL aliquot from each urine sample was sent to the National Center for Environmental Health's (NCEH) laboratory for analysis. The following detailed description of the analysis method used to detect atrazine and its degradation products in urine samples was prepared by Dana Barr and Parinya Panuwet of the NCEH.

The samples were analyzed using the method of Olsson et al.(2004). Urinary creatinine was also measured in the urine samples using a commercially-available enzyme slide technology (Vitros 250 Chemistry System, Ortho-Clinical Diagnostics). A sensitive, selective, and precise automated On-line SPE-HPLC-MS/MS method to measure urinary biomarkers of human exposure to atrazine and its degradation compounds was developed and used for this study (Panuwet et al., 2007, in press). To

date, this method is the most comprehensive and sensitive for measuring multiple atrazine metabolites and is considered to be less labor-intensive and more cost effective than other methods and uses only a small amount of urine, thus preserving sample material and minimizing matrix interferences (Panuwet et al., 2007, in press).

According to this method, "Nine working standard spiking solutions, containing ATZ-OH, DACT, DIA, DEA, and DEAMM, were prepared by serial dilution with methanol of the initial stock solutions to cover the concentration range of 0.5-200 ng/mL equivalent in urine, whereas the concentration range for ATZ and ATZMM was 25-80 ng/mL. The isotope-labeled standard spiking solution was also prepared in methanol, given an approximate concentration of the individual labeled compounds of 12.5 ng/mL.

Quality control (QC) materials were prepared from urine collected from multiple anonymous donors. Urine samples were combined, mixed overnight at 20°C, and then pressure filtered with a 0.45 μm filter capsule (Whatman Inc, Florham Park, NJ, USA). The urine pool was divided into four pools. Three pools were spiked with the target analytes to yield approximate concentrations of 50 ng/mL (QCHigh (QCH)), 10 ng/Ml, (QCMedium (QCM)) and 5 ng/mL (QCLow (QCL)), respectively. The last pool of urine was left as blank urine. This pool was used as matrix material for calibration standards and blanks. All quality control materials including blank urine pool were placed in a freezer at -20 $^{0}$C until use.

The SPE-HPLC was performed on an Agilent 1100 system (Agilent Tech., Waldbronn, Germany) consisting of two quaternary pump, two degassers, an auto sampler with a 900-μL injection loop, and a temperature-stable column compartment. All unknown, blank, and QC samples were prepared in 1.50 mL round bottomed amber

autosampler vials (Agilent Tech., Waldbronn, Germany). A 200 μL aliquot of urine was mixed with 20 μL of internal standard (equivalent to 12.5 ng/mL in urine) and homogenized. Formic acid (0.1 % in water) was added to the sample to bring the volume to 1 mL. The total injection volume was 500 μL. Mass spectrometry analysis was performed on a TSQ Quantum Ultra mass spectrometer (Thermo Electron, San Jose, CA) equipped with an atmospheric pressure chemical ionization (APCI) interface to generate gas phase ions of the target analytes. The mass spectrometer was programmed and controlled using Xcalibur software (Thermo Electron, FL, USA). A total of four precursor/product ion pairs were primarily selected for further optimization in both HPLC and MS/MS quantitative analysis. Ultimately, the best precursor/product ion pair was chosen for the quantification ion and the two next best for confirmation ions. Each labeled internal standard was monitored using one precursor/product ion pair.

The limit of quantification (LOQ) was defined as the lowest standard giving a consistent signal to noise (S/N) ratio of 3. The extraction recovery of the method was determined at two concentrations for each analyte: 5 ng/mL and 25 ng/mL for all target analytes accept ATZ and ATZMM which were 2 ng/mL and 10 ng/mL. The precision of the method was determined by calculating the relative standard deviation (RSD) of repeat measurements of samples from the quality control pools (QCH, QCM, and QCL). Five samples from each of the QC materials were prepared and analyzed every day during a ten-day period, and the results were used to determine the within-day precision and among-day precision. Accuracy was assessed by five replicate analyses of urine spiked at four different concentrations and expressed as the percentage of the expected value. Long-term chemical stability was also tested by spiking blank urine sample with three

different known amounts of standard solution and then stored at -70 $^{0}$C. For the six-month period, the samples were removed from the storage and allowed to reach room temperature. Once samples were prepared according to previous description, the final concentrations of target analytes were approximately 5 ng/mL, 25 ng/mL, and 50 ng/mL respectively for each of five replicates.

Good SPE recoveries were achieved for most of the compounds (>80%) except for DACT. The increased polarity of DACT as compared to our other target analytes probably resulted in decreased sorption time resulting in lower recovery. These data also suggest that alternative SPE sorbents, particularly SCX, might provide an alternative for extraction of DACT from urine samples". The complete description of the atrazine analysis in the urine samples done by NCEH, Atlanta, Georgia is included in Appendix D.

Data Analysis

Atrazine concentrations were reported in nanograms per milliliter of urine (ng/ml) for the urine samples. The atrazine concentrations (ng/ml) are adjusted using each individual's urinary creatinine concentrations expressed in milligrams (mg) per deciliter (dl) of urine to derive creatinine-adjusted urinary atrazine concentrations. The study examined the relationship between farmers and their family member's atrazine exposure, variation in atrazine concentrations over time and factors influencing atrazine contamination in farmer-applied and commercially homes. Descriptive summary statistics were used to describe the central tendency and variance of each variable measured. Regression analysis was used to determine if selected farm and personal practices influenced urine atrazine concentrations. The data collected from the interview

were coded and analyzed to determine if any relationship existed between exposure and selected variables, and which of these variables most influence urine concentrations and exposure. The variables considered included the number of acres of corn grown, the amount of atrazine applied, the number of hours of atrazine application, the number of days of atrazine application, laundering of clothes separately, and if the farmers removed their shoes before entering the homes. The t-statistic was used to compare the difference in pesticide contamination and family exposure between farm homes that did their own spraying and those that hired a commercial applicator for spraying their fields. The urine samples were compared with each other for farmers and their spouses and/or children for farmer-applied and commercial applicator-applied homes for both the planting and non-planting seasons to determine the best indicator of pesticide exposure and to see if any difference exists between the samples. All statistical analyses were performed using SAS system software, version 9.1 (SAS Institute, Inc., Cary, NC). All significance testing was performed with 0.05 as the level of significance.

**Results**

A total of 32 farm families that applied atrazine were recruited in this study. This included ten farm families that had children 15 years old or less living in their household. A total of 73 subjects participated, of which 40 were male and 33 were female. Five out of the 14 children in this study were unavailable to collect the urine sample in the non-planting season. Also, one of the farmer's spouses died during this study, so her urine samples could not be collected in the non-planting season. Evening and morning urine spot samples were collected from each study subject during the planting and non-planting

seasons. The concentration of each atrazine metabolite for each study subject was derived by calculating the mean concentration of the evening and morning urinary sample metabolite concentrations.

Atrazine was applied to the corn fields by either the farmers (10 farm homes) or commercial applicators (22 farm homes). There were no significant differences in the number of acres sprayed, pounds of atrazine applied, or days applied between farm homes where atrazine was applied by farmers themselves and those where it was applied by commercial applicators. However, more hours were spent by the farmers in atrazine application than the commercial applicators did on the study homes' farm fields during the planting season. Only marginal associations were observed in Curwin's study (2005a) for a few of the farm characteristics studied. Time since application and farm size were marginally associated with urinary atrazine levels (Curwin, 2005a). The limit of detection (LOD) for all the atrazine metabolites is 0.1 ng/ml, except for DACT which is 0.5 ng/ml. Concentrations of atrazine and its metabolites above the limit of detection (LOD) in urine samples of farmers, their family members (spouses and children), and the whole family (farmer, spouse and children together) are represented in Tables 3.1, 3.2, and 3.3.

Among the individual atrazine metabolites, the concentrations of Diaminochlorotriazine (DACT) were found in the majority of study subjects in farm homes that did their own pesticide spraying and those that hired a commercial applicator for spraying during both the planting and non-planting seasons. According to Curwin et al. (2005a), for most of the pesticides, farmers who applied the pesticides had significantly higher urinary metabolite levels of that pesticides compared to non-farmers, non-applicators and who hired a commercial applicator. Total atrazine concentrations in

the current study for each individual urine sample were derived by summation of the concentrations of the individual atrazine metabolite concentrations.

Total atrazine= $\sum$ {Conc. of ATZ, ATZ-OH, DACT, DEA, DEAM, DIA, ATZMM}

LOD for Total atrazine (1.1) = LOD for ATZ (0.1) + LOD for ATZ-OH (0.1) + LOD for DACT (0.5) + LOD for DEA (0.1)+ LOD for DEAM (0.1) + LOD for DIA (0.1) + LOD for ATZMM (0.1).

The total atrazine concentrations expressed in ng/ml are adjusted using each individual's urinary creatinine concentrations expressed in milligrams (mg) per deciliter (dl) of urine. Urinary biomonitoring data typically are adjusted to a constant creatinine concentration to correct for variable dilutions among spot samples (Barr et al., 2005).

The total adjusted urinary atrazine concentrations are expressed in micrograms (μg) of atrazine in grams (g) of creatinine. The calculation is as follows:

$$\text{Adjusted urinary atrazine conc. } (\mu g/g) = \frac{\text{Unadjusted urinary atrazine conc. (ng/ml)}}{\text{Urinary creatinine conc. (mg/dl)}} \times 100$$

Hydroxyatrazine was the only atrazine metabolite not detected in the urine samples of any of the study subjects in both the planting and non-planting seasons. Comparing the presence of other urinary atrazine metabolites above the LOD, along with the total atrazine levels above the LOD, during the planting and non-planting seasons, by the type of applicator for the whole family revealed that the number of subjects with total

atrazine above the LOD for the family as a whole was slightly higher in the planting season compared to the non-planting season for self-applied farm families. Among commercially-applied farm families, the number of subjects with total atrazine above the LOD for the family as a whole was slightly higher in the non-planting season compared to the planting season.

**Table 3.1: Concentrations of Atrazine and its Metabolites above the Limit of Detection (LOD) in Urine Samples of the Whole Family**

| | Self-applied Planting season (N=25) | Self-applied Non-planting season (N=23) | Commercially-applied Planting season (N=48) | Commercially -applied Non-planting season (N=44) |
|---|---|---|---|---|
| **Urinary metabolite** | **N (%)** | **N (%)** | **N (%)** | **N (%)** |
| Atrazine | 10 (38) | 1 (4) | 16 (33) | 6 (14) |
| Hydroxyatrazine | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Diaminochlorotriazine | 23 (88) | 19 (82) | 30 (63) | 37 (84) |
| Desethylatrazine | 4 (15) | 3 (13) | 7 (15) | 1 (2) |
| Dea mercapturate | 15 (58) | 8 (35) | 24 (50) | 20 (45) |
| Desisopropylatrazine | 4 (15) | 3 (13) | 5 (10) | 3 (7) |
| Atrazine mercapturate | 6 (23) | 0 (0) | 4 (8) | 0 (0) |
| Total Atrazine | 25 (96) | 20 (87) | 38 (79) | 39 (89) |

Comparing the presence of other urinary atrazine metabolites above the LOD along with the total atrazine levels above the LOD, during the planting and non-planting seasons by the type of applicator for the farmers revealed that the number of subjects with total atrazine above the LOD was similar in both the planting and non-planting season for self-applied farmers and commercially-applied farmers (Table 3.2).

**Table 3.2: Concentrations of Atrazine and its Metabolites above the Limit of Detection (LOD) in Urine Samples of Farmers**

| | Self-applied Planting season (N=10) | Self-applied Non-planting season (N=10) | Commercially-applied Planting season (N=22) | Commercially-applied Non-planting season (N=22) |
|---|---|---|---|---|
| **Urinary metabolite** | **N (%)** | **N (%)** | **N (%)** | **N (%)** |
| Atrazine | 3 (30) | 1 (10) | 7 (32) | 2 (9) |
| Hydroxyatrazine | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Diaminochlorotriazine | 9 (90) | 8 (80) | 13 (59) | 17 (77) |
| Desethylatrazine | 4 (40) | 1 (10) | 1 (5) | 0 (0) |
| Dea mercapturate | 6 (60) | 5 (50) | 10 (45) | 8 (36) |
| Desisopropylatrazine | 1 (10) | 2(20) | 3 (14) | 2 (9) |
| Atrazine mercapturate | 5 (50) | 0 (0) | 1 (5) | 0 (0) |
| Total Atrazine | 9 (90) | 9 (90) | 17 (77) | 18 (82) |

Farmers that applied their own pesticides had atrazine levels above the LOD in their family members' urine more in the planting season compared to the non-planting season. Those farmers that had a commercial applicator apply the pesticides had detectable atrazine in their family members' urine above the LOD more in the non-planting season than the planting season (Table 3.3).

**Table 3.3: Concentrations of Atrazine and its Metabolites above the Limit of Detection (LOD) in Urine Samples of Farmers' Family Members**

| | Self-applied Planting season (N=15) | Self-applied Non-planting season (N=13) | Commercially-applied Planting season (N=26) | Commercially-applied Non-planting season (N=22) |
|---|---|---|---|---|
| **Urinary metabolite** | **N (%)** | **N (%)** | **N (%)** | **N (%)** |
| Atrazine | 7 (47) | 0 (0) | 9 (35) | 4 (18) |
| Hydroxyatrazine | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Diaminochlorotriazine | 14 (93) | 11 (84) | 17 (65) | 20 (91) |
| Desethylatrazine | 0 (0) | 2 (15) | 6 (23) | 1 (5) |
| Dea mercapturate | 9 (60) | 3 (23) | 14 (54) | 12 (55) |
| Desisopropylatrazine | 3 (20) | 1 (8) | 2 (8) | 1 (5) |
| Atrazine mercapturate | 1 (7) | 0 (0) | 3 (12) | 0 (0) |
| Total Atrazine | 15 (100) | 11 (85) | 21 (81) | 21 (95) |

Urinary total atrazine levels above and below the LOD for the whole family during the planting season are marginally significantly different between self and commercially-applied farm families (p- value = 0.05). This is presented in Table 3.4. During the non-planting season, there was no significant difference found between the self-applied and commercially-applied homes for the whole families urinary total atrazine levels above and below the LOD.

**Table 3.4: Comparison of Detectable and Non-Detectable Urinary Total Atrazine Concentrations between Planting and Non-Planting Seasons for Whole Family**

| | **Planting season** | | | |
|---|---|---|---|---|
| | **Self-applied** | **Commercially-applied** | **Chi-Square** | **p-value** |
| **< LOD** | 1 | 10 | 3.84 | 0.05 |
| **> LOD** | 25 | 38 | | |
| **Total** | 26 | 48 | | |
| | **Non-Planting season** | | | |
| | **Self-applied** | **Commercially-applied** | **Chi-Square** | **p-value** |
| **< LOD** | 6 | 9 | 0.19 | 0.5 |
| **> LOD** | 20 | 39 | | |
| **Total** | 26 | 48 | | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

In Table 3.5, urinary total atrazine levels above and below the LOD for farmers during both the planting and non-planting seasons are not significantly different between self and commercially- applied for farm family members (p- value > 0.1).

**Table 3.5: Comparison of Detectable and Non-Detectable Urinary Total Atrazine Concentrations between Planting and Non-Planting Seasons for Farmers**

| | Planting season | | | |
|---|---|---|---|---|
| | **Self-applied** | **Commercially-applied** | **Chi-Square** | **p-value** |
| **< LOD** | 1 | 5 | 0.73 | >0.1 |
| **> LOD** | 9 | 17 | | |
| **Total** | 10 | 22 | | |
| | **Non-Planting season** | | | |
| | **Self-applied** | **Commercially-applied** | **Chi-Square** | **p-value** |
| **< LOD** | 1 | 4 | 0.34 | 0.5 |
| **> LOD** | 9 | 18 | | |
| **Total** | 10 | 22 | | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

Farm and household characteristics have been studied in relation to farmer's urinary total atrazine levels in those who applied their own pesticides and those that hired a commercial applicator during the planting and non-planting seasons. The farm and household characteristics studied were the number of acres of corn sprayed by atrazine, the number of days of atrazine application, the amount of atrazine applied, whether the farmer laundered his clothes separately or with the rest of the family, and if he changed his work clothes and work shoes inside or outside the home (Tables 3.6 & 3.7).

**Table 3.6: Farm and Household Characteristics and Detectable Urinary Total Atrazine in Farmers by Type of Applicator during the Planting Season**

| | Farmer Applicator | | | Commercial Applicator | | |
|---|---|---|---|---|---|---|
| **No. of corn acres sprayed** | **< LOD** | **> LOD** | **p-value** | **< LOD** | **> LOD** | **p-value** |
| < 300 | 1 | 4 | >0.1 | 3 | 10 | >0.5 |
| > 300 | 0 | 5 | | 3 | 6 | |
| **Number of days of atrazine application** | | | | | | |
| 1-4 | 1 | 3 | >0.1 | 3 | 14 | 0.05 |
| > 5 | 0 | 6 | | 3 | 2 | |
| **Amount of atrazine applied in lbs.** | | | | | | |
| < 750 | 1 | 5 | >0.1 | 3 | 10 | >0.5 |
| > 750 | 0 | 4 | | 3 | 6 | |
| **Launder clothes separate from rest of the family** | | | | | | |
| Yes | 0 | 5 | >0.1 | 5 | 9 | >0.1 |
| No | 1 | 4 | | 1 | 7 | |
| **Change work clothes inside the home** | | | | | | |
| Yes | 1 | 6 | >0.1 | 4 | 10 | >0.5 |
| No | 0 | 3 | | 2 | 6 | |
| **Change shoes inside the home** | | | | | | |
| Yes | 1 | 6 | >0.1 | 3 | 8 | >0.5 |
| No | 0 | 3 | | 3 | 8 | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

During the planting and non-planting seasons, farmers' urinary total atrazine levels in those that sprayed pesticides themselves and those that hired a commercial applicator were not statistically significant for the farm and household characteristics studied.

**Table 3.7: Household Characteristics and Detectable Atrazine in Farmers by Type of Applicator during the Non-Planting Season**

| | Farmer Applicator | | | Commercial Applicator | | |
|---|---|---|---|---|---|---|
| **No. of corn acres sprayed** | **< LOD** | **> LOD** | **p-value** | **< LOD** | **> LOD** | **p-value** |
| < 300 | 0 | 5 | >0.1 | 2 | 11 | >0.5 |
| > 300 | 1 | 4 | | 2 | 7 | |
| **Number of days of atrazine application** | | | | | | |
| 1-4 | 1 | 3 | >0.1 | 3 | 14 | >0.5 |
| > 5 | 0 | 6 | | 1 | 4 | |
| **Amount of atrazine applied in lbs.** | | | | | | |
| < 750 | 0 | 6 | >0.1 | 2 | 11 | >0.5 |
| > 750 | 1 | 3 | | 2 | 7 | |
| **Launder clothes separate from rest of the family** | | | | | | |
| Yes | 1 | 4 | >0.1 | 3 | 11 | >0.5 |
| No | 0 | 5 | | 1 | 7 | |
| **Change work clothes inside the home** | | | | | | |
| Yes | 1 | 6 | >0.1 | 2 | 12 | 0.5 |
| No | 0 | 3 | | 2 | 6 | |
| **Change shoes inside the home** | | | | | | |
| Yes | 1 | 6 | >0.1 | 2 | 9 | >0.5 |
| No | 0 | 3 | | 2 | 9 | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

Among the family members of farmers that hired a commercial applicator for pesticide application, those that changed their work clothes inside the homes had marginally significantly higher number of homes with total atrazine concentration greater than LOD compared to those that changed their clothes outside (p-value = 0.05) as seen in Table 3.8. Also, those farmers that had atrazine sprayed by a commercial applicator for one to four days during the planting season had marginally significantly higher number of

homes that had total atrazine above the LOD compared to those that had atrazine sprayed for more than five days (p-value = 0.05).

**Table 3.8: Household Characteristics and Detectable Atrazine in Farmers' Family Members by Type of Applicator during the Planting Season**

| | Farmer-applied Homes | | | Commercial Applicator-applied Homes | | |
|---|---|---|---|---|---|---|
| **No. of corn acres sprayed** | **< LOD** | **> LOD** | **p-value** | **< LOD** | **> LOD** | **p-value** |
| < 300 | 0 | 6 | 1.0 | 1 | 12 | 0.1 |
| > 300 | 0 | 10 | | 4 | 9 | |
| **Number of days of atrazine application** | | | | | | |
| 1-4 | 0 | 10 | 1.0 | 2 | 17 | 0.05 |
| > 5 | 0 | 6 | | 3 | 4 | |
| **Amount of atrazine applied in lbs.** | | | | | | |
| < 750 | 0 | 7 | 1.0 | 1 | 12 | 0.1 |
| > 750 | 0 | 9 | | 4 | 9 | |
| **Launder clothes separate from rest of the family** | | | | | | |
| Yes | 0 | 12 | 1.0 | 4 | 13 | >0.1 |
| No | 0 | 4 | | 1 | 8 | |
| **Change work clothes inside the home** | | | | | | |
| Yes | 0 | 11 | 1.0 | 5 | 12 | 0.05 |
| No | 0 | 5 | | 0 | 9 | |
| **Change shoes inside the home** | | | | | | |
| Yes | 0 | 13 | 1.0 | 5 | 8 | >0.1 |
| No | 0 | 3 | | 0 | 13 | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

During the non-planting season, farmers' family member's urinary total atrazine levels in those that sprayed pesticides themselves and those that hired a commercial

applicator were not statistically significant for the farm and household characteristics studied (Table 3.9).

**Table 3.9: Household Characteristics and Detectable Atrazine in Farmers' Family Members by Type of Applicator during the Non-Planting Season**

| | Farmer-applied Homes | | | Commercial Applicator-applied Homes | | |
|---|---|---|---|---|---|---|
| **No. of corn acres sprayed** | **< LOD** | **> LOD** | **p-value** | **< LOD** | **> LOD** | **p-value** |
| < 300 | 1 | 5 | >0.5 | 0 | 9 | <0.5 |
| > 300 | 1 | 6 | | 1 | 11 | |
| **Number of days of atrazine application** | | | | | | |
| 1-4 | 1 | 6 | >0.5 | 1 | 13 | 0.5 |
| > 5 | 1 | 5 | | 0 | 7 | |
| **Amount of atrazine applied in lbs.** | | | | | | |
| < 750 | 1 | 6 | >0.5 | 0 | 8 | 0.5 |
| > 750 | 1 | 5 | | 1 | 12 | |
| **Launder clothes separate from rest of the family** | | | | | | |
| Yes | 1 | 8 | 0.5 | 1 | 13 | 0.5 |
| No | 1 | 3 | | 0 | 7 | |
| **Change work clothes inside the home** | | | | | | |
| Yes | 2 | 7 | >0.1 | 1 | 13 | 0.5 |
| No | 0 | 4 | | 0 | 7 | |
| **Change shoes inside the home** | | | | | | |
| Yes | 2 | 8 | >0.1 | 1 | 9 | >0.1 |
| No | 0 | 3 | | 0 | 11 | |

*p-value < 0.05 obtained from Chi-Square test is statistically significant

The household and farm characteristics were analyzed for associations with planting and non-planting average urine atrazine concentrations of the farmers and their spouses. PROC GLM was used to identify associations between continuous and

categorical variables of the study homes as presented in Tables 3.10 and 3.11. Among farmers that applied their own pesticides, change of shoes outside the home (GM = 119.9 ng/ml) was significantly higher than those that changed their shoes inside the home (GM = 38.75 ng/ml) when compared with the mean atrazine concentration (GM = 63.09 ng/ml, p-value = 0.01) during the planting season, and changing their work clothes outside the house was significantly higher (GM = 122.62 ng/ml) than changing their work clothes inside the homes (GM = 37.58 ng/ml) compared with the mean atrazine concentration in the planting season (GM = 63.09ng/ml, p-value=0.007). Among farmers who applied their own pesticides, the number of acres of corn fields, amount of atrazine applied, and the number of days and hours of atrazine application were not significantly associated with the mean atrazine concentration of farm homes in both the planting and non-planting seasons (Table 3.10). Among farmers who had a commercial applicator apply pesticides on their fields, the number of hours of atrazine application were positively associated with the planting season (GM =24.83 ng/ml, p-value = 0.001).

Among farmer's spouses who had a commercial applicator apply pesticides on their fields, changing their work clothes outside the house was significantly higher than changing their work clothes inside the homes compared with the mean atrazine concentration in the planting and non-planting seasons (p-value < 0.05) as presented in Table 3.11. All the other variables studied were not significantly associated with the spouses' total urinary atrazine concentrations.

**Table 3.10: Univariate Associations of Household and Farm Characteristics with Farmers' Total Urinary Atrazine Concentrations (ng/ml) in Planting and Non-Planting Seasons**

| | Farmers of Self-Application Homes | | Farmers of Commercial-Application Homes | |
|---|---|---|---|---|
| **Characteristic** | **Planting (p-value)** | **Non-Planting (p-value)** | **Planting (p-value)** | **Non-Planting (p-value)** |
| Age of home | 0.99 | 0.94 | 0.61 | 0.43 |
| Age of carpet | 0.25 | 0.82 | 0.47 | 0.39 |
| Number of corn acres sprayed with atrazine | 0.48 | 0.12 | 0.97 | 0.33 |
| Number of pounds of atrazine applied | 0.48 | 0.12 | 0.98 | 0.33 |
| Number of days of atrazine application | 0.96 | 0.68 | 0.40 | 0.29 |
| Number of hours of atrazine application per day | 0.07 | 0.33 | 0.001* | 0.21 |
| Any pesticide applied inside home within past year | 0.98 | 0.42 | 0.30 | 0.48 |
| Lawns treated with any pesticide within past year | 0.13 | 0.69 | 0.80 | 0.21 |
| Garden treated with any pesticide within past year | 0.40 | 0.63 | 0.45 | 0.70 |
| Carpet vacuumed at least once a week | 0.57 | 0.46 | 0.97 | 0.78 |
| Presence of Doormats | 0.91 | 0.74 | 0.58 | 0.88 |
| Presence of Pets | 0.22 | 0.30 | 0.06 | 0.37 |
| Launder clothes separate from rest of the family | 0.79 | 0.41 | 0.71 | 0.20 |
| Change work clothes inside the home | 0.007* | 0.09 | 0.85 | 0.44 |
| Change shoes inside the home | 0.01* | 0.12 | 0.17 | 0.24 |

* p-value < 0.05 obtained from PROC GLM is statistically significant

**Table 3.11: Univariate Associations of Household and Farm Characteristics with Farmer's Spouses' Total Urinary Atrazine Concentrations (ng/ml) in Planting and Non-Planting Seasons**

| | Spouses in Self-Application Homes | | Spouses in Commercial-Application Homes | |
|---|---|---|---|---|
| **Characteristic** | **Planting (p-value)** | **Non-Planting (p-value)** | **Planting (p-value)** | **Non-Planting (p-value)** |
| Age of home | 0.92 | 0.95 | 0.71 | 0.35 |
| Age of carpet | 0.07 | 0.01* | 0.98 | 0.35 |
| Number of corn acres sprayed with atrazine | 0.20 | 0.85 | 0.89 | 0.98 |
| Number of pounds of atrazine applied | 0.20 | 0.87 | 0.89 | 0.98 |
| Number of days of atrazine application | 0.67 | 0.56 | 0.43 | 0.15 |
| Number of hours of atrazine application per day | 0.21 | 0.05 | 0.93 | 0.19 |
| Any pesticide applied inside home within past year | 0.53 | 0.40 | 0.75 | 0.62 |
| Lawns treated with any pesticide within past year | 0.65 | 0.11 | 0.34 | 0.75 |
| Garden treated with any pesticide within past year | 0.22 | 0.77 | 0.44 | 0.73 |
| Carpet vacuumed at least once a week | 0.34 | 0.26 | 0.48 | 0.87 |
| Presence of Doormats | 0.94 | 0.40 | 0.72 | 0.68 |
| Presence of Pets | 0.68 | 0.91 | 0.67 | 0.30 |
| Launder clothes separate from rest of the family | 0.86 | 0.40 | 0.62 | 0.72 |
| Change work clothes inside the home | 0.04* | 0.001* | 0.98 | 0.20 |
| Change shoes inside the home | 0.95 | 0.24 | 0.73 | 0.58 |

* p-value < 0.05 obtained from PROC GLM is statistically significant

Urinary total atrazine concentrations measured in the farmers, their spouses, and their children were compared between those farm homes that had their own pesticide

application and those that hired a commercial applicator during both the planting and non-planting seasons (Table 3.12). The total atrazine concentrations in urine are expressed as nanograms (ng) of atrazine per milliliter (ml) of urine.

The mean total atrazine concentration of the farmers' family members (spouses and children together) during the non-planting season (mean = 44 ng/ml) is marginally significantly higher than those in the planting season (mean =19 ng/ml, p-value = 0.06) among those that self-applied their pesticides. The mean total atrazine concentrations of the farmer, the farmer and family members together (whole family) and the farmers' children in the planting season were not statistically significantly different from those in the non-planting season among those that applied the pesticides themselves. The mean total atrazine concentration of the farmers in the planting season (mean = 63 ng/ml) is significantly higher than the mean concentration of the family members (mean = 19 ng/ml) among those that self-applied their pesticides (p-value = 0.003).

Among the farm families that hired a commercial applicator for pesticide application, the mean total atrazine concentrations of the farmer, their family members, the farmer and family members together (whole family) and the farmers' children in the planting season were not statistically significantly different from those in the non-planting season. The mean total atrazine concentration of farmers that self-applied their pesticides in the planting season (mean = 63 ng/ml) is significantly higher than that of farmers that hired a commercial applicator in the planting season (mean = 24 ng/ml; p-value=0.01). This concurs with Curwin's finding that farmers who applied pesticides themselves had significantly higher urinary concentrations of those pesticides compared to those that hired a commercial applicator (Curwin et al., 2005a).

**Table 3.12: Total Atrazine Concentrations during Planting and Non-Planting Seasons expressed in ng/ml**

| | Self-applied Planting season | Self-applied Non-planting season | p-value |
|---|---|---|---|
| **Subjects** | **Mean (SD)** | **Mean (SD)** | |
| **Farmer and family members** | 37 (40.5) | 39 (41.2) | 0.84 |
| **Farmer** | 63 (52.5)[a] | 32 (30.3) | 0.12 |
| **Family members** | 19 (14.7)[c] | 44 (48.6)[a, d] | 0.06 |
| **Children** | 20 (14.4) | 32 (20.3) | 0.28 |
| | **Commercially-applied Planting season** | **Commercially-applied Non-planting season** | **p-value** |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | |
| **Farmer and family members** | 29 (37.9) | 29 (23) | 0.94 |
| **Farmer** | 24 (31.6)[c, b] | 34 (26.6) | 0.3 |
| **Family members** | 34 (42.8) | 24 (18.1) | 0.34 |
| **Children** | 47 (55.4) | 30 (33.3) | 0.55 |

[a, b, c, d] Means are provided in ng/ml where those with the same letter are not significantly different from each other

*p-value < 0.05 obtained from paired t-test is statistically significant

Among farmers that applied their own pesticides, there was no significant difference between the mean creatinine-adjusted total atrazine concentrations of the farmer, their family members, the farmer and family members together (whole family), and the farmers' children in the planting season and in the non-planting season (Table 3.13). Among those farmers that hired a commercial applicator for pesticide application, the mean creatinine- adjusted total atrazine concentration of the farmers (mean = 39 μg/g) in the non-planting season is higher than that of farmers (mean = 23 μg/g) in the planting season (p-value = 0.03).

**Table 3.13: Creatinine-Adjusted Total Atrazine Concentrations during Planting and Non-Planting Seasons expressed in μg/g**

| | Self-applied Planting season | Self-applied Non-planting season | p-value |
|---|---|---|---|
| **Subjects** | **Mean (SD)** | **Mean (SD)** | |
| **Farmer and family members** | 44 (36.68) | 53 (55.04) | 0.53 |
| **Farmer** | 64 (47.01)[c] | 45 (38.4) | 0.33 |
| **Family members** | 32 (21.17) | 59 (65.96) | 0.13 |
| **Children** | 39 (28.39) | 47 (28.96) | 0.65 |
| | **Commercially-applied Planting season** | **Commercially-applied Non-planting season** | **p-value** |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | |
| **Farmer and family members** | 31 (30.55) | 38 (22.37) | 0.17 |
| **Farmer** | 23 (23.5)[a] | 39 (25.4)[b, c] | 0.03 |
| **Family members** | 37 (34.56) | 37 (19.34) | 0.97 |
| **Children** | 41 (36.16) | 32 (21.38) | 0.62 |

[a, b, c] Means with the same letter are not significantly different from each other

*p-value < 0.05 obtained from paired t-test is statistically significant

Among farmers, the urinary creatinine-adjusted total atrazine concentration of those that self-applied their pesticides in the planting season (mean = 64 μg/g) is significantly higher than those that hired a commercial applicator for pesticide application in the non-planting season (mean = 23 μg/g; p-value = 0.002). This shows that the unadjusted urinary atrazine concentration pattern holds for creatinine adjusted urinary atrazine concentrations for the most part in this study. This is comparable to Curwin's findings about the use of creatinine adjusted urinary values (Curwin et al., 2005a).

**Discussion**

This study found that the urine samples collected from farmers, their spouses, and their children had detectable concentrations of atrazine and/or its metabolites in the planting and non-planting seasons. This finding supports the findings from Curwin's study (2007) that farm family members had higher urinary pesticide levels for atrazine compared to non-farm family members. Among children, only atrazine out of all the pesticides under study was significantly higher (Curwin et al., 2007). The farmers in the current study differed in pesticide application. Some of the farmers applied their own pesticides to the crop fields, whereas the others hired a commercial applicator to apply the pesticides. Curwin et al. (2007) stated that the principal farmer (father) of each farm home would have had a possibility of greater pesticide exposure compared to non-farm fathers. Urinary total atrazine levels above and below the LOD for farmers during both the planting and non-planting seasons are not significantly different between self-applied and commercially-applied farm families. This finding suggests that there are exposures to atrazine not only during the planting season when the farmer is most likely to be directly exposed during the application event, but also during the non-planting season when there is no atrazine application. This is suggestive of take-home pesticide exposure as has been observed in previous studies by Curwin et al. (2005a, 2007). Comparison of the total urinary atrazine concentrations for each study subject revealed that there was no significant difference between the concentrations in the planting and non-planting seasons for most of the study subjects. Family members (spouses and children) are the ones to be most directly affected by potential take-home pesticide exposures, since a lack of occupational exposure to atrazine in the planting season might still cause an exposure

in both the planting and non-planting seasons at home. However, the family members of farmers that self-applied atrazine had significantly higher concentrations in the non-planting season compared to the planting season. This is not seen among the family members of the homes that were commercially applied. The creatinine adjusted atrazine concentrations of family members did not differ significantly. The higher non-planting season concentrations could be because of the possibility of contribution of exposures through other routes not researched as part of this study.

Mean total urinary atrazine concentrations unadjusted for urinary creatinine expressed in ng/ml did not significantly decrease over a period of six months. Also, the creatinine-adjusted urinary total atrazine concentrations expressed in micrograms of atrazine per gram of creatinine did not significantly decrease over a period of six months. The absence of a significant decrease in urinary atrazine concentrations in farm families from the planting to non-planting seasons suggests the persistence of exposures to this herbicide in farm homes irrespective of its application. This take-home pathway has been supported through findings in previous study where the application of a pesticide by the father appeared to influence farm family member's exposures (Curwin et al., 2007). Among the individual atrazine metabolites in urine samples of farmers, their spouses and children, the concentrations of Diaminochlorotriazine (DACT) were found in the majority of study subjects in farm homes that did their own pesticide spraying and those that hired a commercial applicator for spraying during both the planting and non-planting seasons. Comparing the presence of total urinary atrazine levels above the LOD in farmers, their spouses and children, and the whole family during the planting and non-planting seasons by the type of applicator (pesticides self –applied or commercially-

applied) revealed that the number of subjects with total atrazine above the LOD was similar in both the planting and non-planting seasons. This finding further emphasizes the persistence of atrazine and the presence of exposures in both seasons. This can be compared to the study of the use and subsequent disuse of chlorpyrifos in residential applications still causing ever-present concentrations of this pesticide in household environments (CDC, 2003; Curwin et al., 2005b; Fenske et al., 2002).

Several household and farm characteristics were studied for association with planting and non-planting urinary atrazine concentrations for the farmers and their spouses. Children were not included in this analysis due to their small sample size. Most of the variables studied were not significant. The higher urinary concentrations of atrazine among farmers and their spouses when changed their shoes and work clothes outside compared with those that changed inside can be explained by the huge amounts of atrazine applied on these crop fields that could significantly increase the likelihood of this pesticide being brought into the homes and being detected inside farm homes. Since most of the variables were not significant, multivariate analysis was not performed.

During the planting and non-planting seasons, farmers' urinary total atrazine levels in those that sprayed pesticides themselves and those that hired a commercial applicator were not significantly different for the farm and household characteristics studied. Whether the farm homes self-apply or commercially-apply, exposure to atrazine seems to be influenced equally in both groups. Among the family members of farmers that hired a commercial applicator for pesticide application, those that changed their work clothes inside the homes, and those that had atrazine sprayed by a commercial applicator for one to four days during the planting season had significantly higher number of homes

with total atrazine concentration greater than LOD compared to those that changed their clothes outside and those that sprayed for more than five days. Changing shoes inside the home might track in dust and dirt that may contain atrazine which may in turn contaminate the home environment causing an exposure to the farmers and their family members. The spraying of atrazine for one to four days leading to urinary atrazine levels above the LOD can be related to the amount of atrazine sprayed in those days and the continuity of spraying that can lead to continuous exposure in farmers and their family members inside homes. The amount of atrazine applied and the number of acres sprayed did not significantly differ between the study subjects of farmer-applied and commercially-applied homes for urinary atrazine concentrations above and below the LOD.

Some of the strengths of this study are these results indicating that homes that did their own atrazine application are not significantly different than those that had a commercial application. This finding is contrary to the common belief that farm homes that hire a commercial applicator to spray the pesticides on their fields would not be exposed to pesticides applied on the fields. This study also reveals that the whole family average atrazine concentrations did not significantly decrease over a period of one year for farm homes that did their own atrazine application and those that hired a commercial applicator. The positive detection of atrazine and its metabolites in the urine samples of almost all the study subjects in both the planting and non-planting seasons indicates that the herbicide atrazine causes exposure to farm families inside their homes irrespective of their involvement in pesticide application. The limitations in this study are that not all exposure pathways have been studied which could fully account for all the exposures that

have taken place inside farm homes and the presence of urinary atrazine concentrations in the non-planting season. Dermal exposure along with ingestion exposure characterization can better explain the trends of these absorbed doses of atrazine in farm families.

**Conclusion**

This study found that all study subjects in all homes located on a farm, whether they self-apply or commercially-apply had atrazine exposure inside the homes. The whole family average total urinary atrazine concentrations for both the self-applied and commercially-applied homes were not statistically significantly different between the planting and non-planting season. Urinary total atrazine concentrations did not decrease significantly over a period of one year in homes applied their own pesticides and those that hired a commercial applicator for pesticide application. It is the amount of atrazine applied continuously and persistence of this herbicide inside farm homes that cause the potential exposures to not only the farmers, but also their spouses, children, and anyone living in their household which can lead to the bioavailability of this herbicide atrazine in farm families both in the planting and non-planting seasons.

# CHAPTER 4

# ASSOCIATION BETWEEN VACUUM DUST AND URINARY ATRAZINE CONCENTRATIONS: PESTICIDE DOSE ESTIMATES IN FARM FAMILIES OVER TIME

## Introduction

In recent years there has been a significant increase in the use of herbicides and pesticides in the production of corn soybeans, and other agricultural crops (NCFAP, 2003). The increased reliability on the efficiency and efficacy of these substances has caused many environmental threats, affecting not only the ecosystem in general but the human population and its health as well (Porter et al., 1999). The risk of adverse health effects from pesticide exposures can be studied through an understanding of the agricultural chemicals used, their routes of exposure, toxicity, duration of exposure, and absorbed doses. Environmental and biological measurements are frequently used to characterize and assess exposures. The usefulness of both environmental and biological data has been demonstrated in a study reported in 1997 by Shealy et al. Residues from recently sprayed pesticides can show up in dust, however, dust can also act as a reservoir for pesticides used on a long-term basis and even for pesticides that are no longer used but are environmentally persistent (Bradman et al., 1997; Colt et al., 1998; Lewis et al., 1994; Lewis et al., 2001).

Environmental measurements are usually easy to obtain and can be good indicators of the actual intake of a pesticide, especially if multiple intake routes are evaluated. However, the most direct indicator of the intake of toxicants during an exposure is the measurement of the body burden or internal dose of toxicants or their

metabolites in blood, urine, or tissues (Shealy et al., 1997). Shealy also demonstrated the usefulness of both environmental and biological data in evaluating environmental exposures. In his study, a strong correlation was observed between environmental carbaryl (carbamate insecticide) measurements with serum and urinary 1-naphthol (metabolite of carbaryl) measurements in farmer applicators and their families.

Occupational exposures may allow for more exposure to atrazine itself while environmental exposures are likely dominated by exposure to the dealkylated environmental degradates. Thus, measurement of multiple potential metabolites is necessary to accurately assess exposure to atrazine and its related degradates (Panuwet et al., 2007 in press).

In 2001, NIOSH conducted a study of in-home pesticide levels in 25 farm and 25 non-farm homes (Curwin et al., 2005b). Air, surface wipe, and vacuum dust samples were collected from these homes and analyzed for the presence of several pesticides: acetochlor, alachlor, chlorpyrifos, 2,4-D, atrazine, metolachlor, and glyphosate. In the same year, NIOSH conducted a study of urinary pesticide levels among farmers and non-farmers in Iowa (Curwin et al., 2005a). Urine samples were collected from fathers, mothers, and their children on two occasions one month apart and analyzed for the presence of the above pesticides. This study revealed that detection of atrazine in the hand wipes was significantly associated with urinary levels of atrazine above the median (p-value $< 0.01$). The urinary pesticide metabolite levels were correlated among the family members, for most of the pesticides studied (Curwin et al., 2007). This study also revealed that in farm homes, father's urinary pesticide levels were correlated with the mother's and children's levels. Children's estimated doses were calculated for atrazine,

metolachlor, chlorpyrifos, and glyphosate and compared to EPA reference doses (Curwin et al., submitted to Environmental Health Perspectives). It was found that the doses from farm children were higher than from those of non-farm children for all pesticides except glyphosate. However, none of the doses exceeded the EPA chronic reference values for atrazine, metolachlor, and glyphosate. All of the doses for chlorpyrifos exceeded the EPA chronic population adjusted reference value in this study.

Although very informative, these studies were not able to clearly demonstrate if any significant association exists over a period of one year and if the estimated atrazine doses change over a period of time in reference to the EPA levels. The results of Curwin's study laid the foundation for the current study in collecting environmental and biological data to more fully learn how families are exposed to pesticides through in-home migration and to study any correlation between dust samples collected from farm homes and urine samples collected from the farm families of those homes, and the estimated atrazine doses. The objective of the current study is to provide an analysis and evaluation of the correlation between the atrazine levels in the dust samples and the atrazine metabolite levels in the urine samples; study the changes in these associations over time; study the comparisons of these results with toxicological data; and assess potential farm family health risks.

**Methods**

The study involved sampling surveys of 32 farm families and their homes from Cedar, Iowa, and Johnson counties in Iowa, which use the target pesticide atrazine. Ten of the farm families had children. The families were surveyed on two separate occasions during the study year 2005: initially during the planting season (April-June) and after the

end of the planting season (November-December). Environmental pesticide levels were assessed by collecting dust samples from four locations within the homes: entryway, living room, master bedroom, and kitchen and analyzed for atrazine. During each home visit both spouses and any children in the home were asked to provide two spot-urine samples, one in the evening of the day of the visit, and one the following morning. These urine samples were analyzed for atrazine. A detailed description of the study participant recruitment process along with dust and urine sampling methodologies is described in chapters 2 and 3 respectively.

Sample Methods for Dust

Dust samples were collected from carpets using a high-volume surface sampler (HVS-3, Cascade Stamp Sampling Systems) using the American Society for Testing Material (ASTM) Standard Practice for Collection of Dust from Carpeted Floors for Chemical Analysis (ASTM D 5438-94, 2000). The dust samples were analyzed using gas chromatography/mass spectrometry in the multiple ion detection mode (GC/MS/MID) at Battelle Memorial Institute (Columbus, Ohio). The description of the laboratory analysis for atrazine in the dust samples is included in Appendix C.

Sample Methods for Urine

The urine samples were sent to the National Center for Environmental Health's (NCEH) laboratory for atrazine analysis using the method of Olsson et al. A sensitive, selective, and precise automated on-line solid phase extraction-isotope dilution-high performance liquid chromatography-tandem mass spectrometry (SPE-HPLC-MS/MS)

method to measure urinary biomarkers of human exposure to atrazine and its degradation compounds was used for this study. The atrazine analysis in the urine samples was performed by NCEH, Atlanta, Georgia, a description of which is included in Appendix D.

Atrazine Dose Estimates

The absorbed daily dose (ADD) for atrazine was calculated using the formula adopted from Curwin's study (Curwin et al., submitted):

$$ADD(mg/kg/day) = \left\{ \frac{(C)(Cn)(CF)(R_{mw})}{BW} \right\}$$

where;

C = concentration of atrazine or its metabolite in urine per gram creatinine (μg/g),

Cn = calculated mass of creatinine excreted per day (g/day),

CF = correction factor,

$R_{mw}$ = ratio of parent pesticide and its metabolite molecular weight (0.63, for atrazine)

BW = body weight (kg)

Total daily (24-hour) excretion of creatinine (Cn) was calculated using:

$$Cn(g/day) = \left( \frac{CnER \times 1440min/day}{1.73} \right) \times BSA \times \left( \frac{1g}{1000mg} \right)$$

where;

Cn ER = creatinine urinary excretion rate in mg/min per 1.73 $m^2$ body surface area,
BSA = body surface area ($m^2$)

Creatinine urinary excretion rate was calculated as a function of age (Shull et al., 1978):

$$\text{Cn ER} = 0.035 \times \text{age (yrs)} + 0.236$$

BSA was calculated as a function of height and weight (Morsteller et al., 1987):

$$\text{BSA } (m^2) = \left[\frac{\text{ht(cm)} \times \text{wt(kg)}}{3600}\right]^{0.5}$$

Due to incomplete excretion of pesticides in urine, correction factors were used in calculating the absorbed doses for atrazine (Curwin et al., submitted). According to Timchalk et al. (1990), 67% of atrazine is excreted in urine. Among the excreted metabolites of atrazine, atrazine mercapturate accounts for 80% (Buchholtz et al., 1999). This results in a correction factor of (1/0.67)/0.8 = 1.9 (Curwin et al., submitted).

Data Analysis

Atrazine concentrations were reported in nanograms per gram of dust (ng/g) for the dust vacuum samples and in nanograms per milliliter of urine (ng/ml) for the urine samples. The study examined the relationship between home pesticide contamination and farmer and his family members' urinary atrazine concentrations, along with variation in atrazine concentrations over time. Pearson's correlation was used to determine if home atrazine contamination was associated with the urinary atrazine concentrations of the farmers and their family members. Atrazine dust concentrations from all the four locations in the home were studied for an association with unadjusted and creatinine-

adjusted urinary atrazine concentrations for farmer-applied and commercial applicator-applied homes for both the planting and non-planting seasons. The urinary atrazine concentrations of the evening and morning urinary samples of the farmers and their family members were studied to observe any decrease in the urinary atrazine levels indicative of the excretion rate of atrazine from the exposed individuals. The t-statistic was used to compare the difference between the evening and morning urinary atrazine concentrations, and to compare the absorbed doses in both the planting and non-planting seasons. All statistical analyses were performed using SAS system software, version 9.1 (SAS Institute, Inc., Cary, NC). All significance testing was performed at 0.05 as the level of significance except for correlation analysis which was performed at 0.10 in order to accommodate the small sample size of farm families in this study analysis and to identify any correlation between dust and urinary atrazine concentrations.

**Results**

Evening and morning urine spot samples were collected from each study subject during the planting and non-planting seasons. The concentration of each atrazine metabolite for each study subject was derived by calculating the mean concentration of the evening and morning urinary sample metabolite concentrations. Atrazine was applied to the corn fields by either the farmers (10 farm homes) or commercial applicators (22 farm homes).

Total atrazine concentrations of the evening and morning voids unadjusted for urinary creatinine values for both the planting and non-planting seasons by the type of applicator are expressed in Table 4.1. The total urinary atrazine concentration of the

evening and morning samples is computed for the farmer and his family as a whole, the farmer only, and the spouse and children if any. There was no statistically significant difference observed between the evening and morning total atrazine concentrations during both the planting and non-planting seasons for self-applied and commercially-applied farm homes.

**Table 4.1: Total Urinary Atrazine Concentrations of the Evening and Morning Collections during Planting and Non-Planting Seasons**

| | Self-applied Planting season | | Self-applied Non-planting season | |
|---|---|---|---|---|
| | Evening | Morning | Evening | Morning |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Farmer, spouse and children** | 47 (62.2) | 27 (23.8) | 33 (34.3) | 45 (68.1) |
| **Farmer** | 85 (85.3) | 42 (25.9) | 23 (27.2) | 42 (52.1) |
| **Spouse and children** | 22 (16) | 17 (16.9) | 42 (38) | 47 (80.4) |
| | **Commercially-applied Planting season** | | **Commercially-applied Non-planting season** | |
| | **Evening** | **Morning** | **Evening** | **Morning** |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Farmer, spouse and children** | 26 (34.1) | 33 (51) | 26 (21.3) | 32 (38.3) |
| **Farmer** | 25 (35.5) | 24 (36.4) | 28 (21.2) | 39 (50.2) |
| **Spouse and children** | 26 (33.7) | 41 (60.4) | 25 (21.9) | 24 (18.8) |

Note: Means were compared using a paired t-test and are not significantly different from each other based on p-value $< 0.05$

Urinary atrazine concentrations expressed in ng/ml

Creatinine-adjusted total atrazine concentrations of the evening and morning voids for both the planting and non-planting seasons by the type of applicator are

expressed in Table 4.2. This analysis also reveals that there was no statistically significant difference observed between the evening and morning total atrazine concentrations during both the planting and non-planting seasons for self-applied and commercially-applied farm homes.

**Table 4.2: Creatinine-Adjusted Total Urinary Atrazine Concentrations of the Evening and Morning Collections during Planting and Non-Planting Seasons**

| | Self applied Planting season | | Self applied Non-planting season | |
|---|---|---|---|---|
| | Evening | Morning | Evening | Morning |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Farmer, spouse and children** | 55 (55.2) | 34 (29.2) | 50 (43.5) | 55 (86) |
| **Farmer** | 84 (74.5) | 43 (33) | 42 (47.8) | 47 (48.5) |
| **Spouse and children** | 35 (25.3) | 28 (25.8) | 57 (40.6) | 61 (108.1) |
| | **Commercially applied Planting season** | | **Commercially applied Non-planting season** | |
| | Evening | Morning | Evening | Morning |
| **Subjects** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Farmer, spouse and children** | 28 (32) | 33 (40.7) | 36 (26.9) | 41 (34.2) |
| **Farmer** | 22 (25.3) | 24 (31.8) | 33 (23.9) | 45 (43.6) |
| **Spouse and children** | 34 (36.2) | 40 (46.3) | 38 (30) | 36 (21.3) |

Note: Means were compared using a paired t-test and are not significantly different From each other based on p-value < 0.05

Urinary atrazine concentrations expressed in μg/g

Estimated correlation coefficients using parametric measures to study the correlation between vacuum dust atrazine concentrations from the four locations of each home, the house average dust atrazine concentration, and unadjusted and creatinine-

adjusted total urinary atrazine concentrations of the farmers and their families are represented in Tables 4.3 and 4.4.

Among homes that hired a commercial applicator, the total urinary atrazine concentration in the non-planting season (mean = 28.38 ng/ml) was marginally positively correlated with the entryway, kitchen, and house average dust atrazine concentrations (r = 0.27, 0.29, and 0.26 respectively; p-value < 0.10).

**Table 4.3: Association between Dust Atrazine Concentrations and Total Urine Atrazine Concentrations based on Type of Applicator**

| | Self Application | | Commercial Application | |
|---|---|---|---|---|
| | **Planting Urine Conc.** | **Non-planting Urine Conc.** | **Planting Urine Conc.** | **Non-planting Urine Conc.** |
| | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** |
| **Entryway** | 0.26 (0.20) | 0.25 (0.24) | 0.11 (0.46) | 0.27* (0.08) |
| **Living Room** | 0.22 (0.28) | 0.24 (0.26) | 0.02 (0.85) | 0.19 (0.22) |
| **Master Bedroom** | 0.14 (0.49) | 0.27 (0.20) | -0.01 (0.90) | 0.22 (0.14) |
| **Kitchen** | 0.09 (0.64) | 0.14 (0.49) | -0.005 (0.97) | 0.29* (0.05) |
| **House Avg.** | 0.20 (0.32) | 0.21 (0.31) | -0.01 (0.93) | 0.26* (0.08) |

*p-value considered borderline statistically significant (between 0.05 and < 0.10)

Among homes that self-applied their pesticides, during the planting season, the creatinine-adjusted total urinary atrazine concentration (mean = 44.3 μg/g) was positively correlated with dust atrazine concentration from the living room (r = 0.41, p-value = 0.03).

**Table 4.4: Association between Dust Atrazine Concentrations and Creatinine-Adjusted Total Urine Atrazine Concentrations based on Type of Applicator**

| | Self Application | | Commercial Application | |
|---|---|---|---|---|
| | Planting Urine Conc. | Non-planting Urine Conc. | Planting Urine Conc. | Non-planting Urine Conc. |
| | Pearson Corr. Coeff. (p-value) | Pearson Corr. Coeff. (p-value) | Pearson Corr. Coeff. (p-value) | Pearson Corr. Coeff. (p-value) |
| **Entryway** | 0.29 (0.14) | 0.27 (0.21) | 0.14 (0.33) | 0.20 (0.18) |
| **Living Room** | 0.41* (0.03) | 0.30 (0.15) | 0.09 (0.54) | 0.1 (0.52) |
| **Master Bedroom** | 0.04 (0.82) | 0.28 (0.18) | 0.01 (0.94) | 0.11 (0.47) |
| **Kitchen** | 0.20 (0.31) | 0.19 (0.37) | 0.01 (0.93) | 0.16 (0.28) |
| **House Avg.** | 0.27 (0.18) | 0.25 (0.24) | 0.00003 (0.99) | 0.15 (0.32) |

*p-value < 0.05 and considered statistically significant

Correlation coefficients among family members of those that self-applied their pesticides and those that applied them commercially during the planting and non-planting seasons are presented in Tables 4.5, 4.6 and 4.7.

**Table 4.5: Association between Dust Atrazine Concentrations and Total Urine Atrazine Concentrations for the Planting Season by Type of Applicator**

| | Farmer Applicator | | | Commercial Applicator | | |
|---|---|---|---|---|---|---|
| | Farmer | Wife | Child | Farmer | Wife | Child |
| | Pearson Corr. (p-value) | Pearson Corr. (p-value) | Pearson Corr. (p-value) | Pearson Corr. (p-value) | Pearson Corr. (p-value) | Pearson Corr. (p-value) |
| **Entryway** | 0.52 (0.11) | 0.17 (0.64) | -0.15 (0.76) | 0.31 (0.16) | 0.06 (0.79) | -0.37 (0.40) |
| **Living Room** | 0.34 (0.32) | 0.26 (0.49) | 0.24 (0.64) | 0.24 (0.29) | -0.03 (0.88) | -0.58 (0.16) |
| **Master Bedroom** | 0.24 (0.51) | -0.23 (0.57) | 0.61 (0.19) | 0.15 (0.48) | 0.05 (0.83) | -0.55 (0.19) |
| **Kitchen** | 0.3 (0.39) | -0.22 (0.55) | -0.57 (0.23) | 0.23 (0.32) | -0.02 (0.91) | -0.41 (0.36) |
| **House Avg.** | 0.42 (0.22) | -0.02 (0.94) | -0.18 (0.72) | 0.21 (0.34) | -0.02 (0.92) | -0.47 (0.28) |

During the non-planting season, among those homes that hired a commercial applicator for pesticide application, the total urinary atrazine concentrations of the farmer's spouses (mean = 22.48 ng/ml) was highly correlated with the vacuum dust concentration in the master bedroom (r = 0.51, p-value = 0.03) as shown in Table 11. Also, the children's total urinary atrazine concentrations were marginally correlated with the vacuum dust atrazine concentrations of all the locations and house average (p-value = 0.09).

**Table 4.6: Association between Dust Atrazine Concentrations and Total Urine Atrazine Concentrations for the Non-Planting Season by Type of Applicator**

| | Self Application | | | Commercial Application | | |
|---|---|---|---|---|---|---|
| | **Farmer** | **Wife** | **Child** | **Farmer** | **Wife** | **Child** |
| | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** |
| **Entryway** | 0.14 (0.68) | 0.45 (0.22) | -0.09 (0.90) | 0.16 (0.48) | 0.35 (0.17) | 0.80** (0.09) |
| **Living Room** | 0.06 (0.85) | 0.41 (0.27) | 0.29 (0.70) | 0.02 (0.91) | 0.07 (0.77) | 0.81** (0.09) |
| **Master Bedroom** | 0.45 (0.18) | 0.26 (0.48) | 0.35 (0.64) | -0.06 (0.78) | 0.51* (0.03) | 0.8** (0.09) |
| **Kitchen** | 0.22 (0.53) | 0.30 (0.42) | -0.52 (0.47) | 0.08 (0.71) | 0.4 (0.11) | 0.8** (0.09) |
| **House Avg.** | 0.18 (0.61) | 0.41 (0.26) | -0.4 (0.59) | 0.06 (0.77) | 0.34 (0.19) | 0.81** (0.09) |

*p-value < 0.05 and considered statistically significant

**p-value considered borderline statistically significant (between 0.05 and < 0.10)

Irrespective of the applicator type (Table 4.7), during the planting season, the farmers' total urinary atrazine concentration (mean = 36.54 ng/ml) is strongly positively correlated with the vacuum dust concentrations in the entryway (r = 0.49, p-value =

0.005), the kitchen (r = 0.36, p-value = 0.04), and the average house dust concentration (r = 0.45, p-value = 0.01). During the non-planting season, the wife's total urinary atrazine concentration (mean = 31.87 ng/ml) was highly correlated with the vacuum dust concentrations in the entryway (r = 0.44, p-value = 0.02) and the average house dust concentration (r = 0.41, p-value = 0.04). In the same season, the children's total urinary atrazine concentration (mean = 31.21 ng/ml) was positively correlated with the vacuum dust concentrations in the living room (r = 0.72, p-value = 0.02), and the master bedroom (r = 0.72, p-value = 0.02).

**Table 4.7: Association between Dust Atrazine Concentrations and Total Urine Atrazine Concentrations for the Planting Season and Non-Planting Seasons**

| | Planting season | | | Non-planting season | | |
|---|---|---|---|---|---|---|
| | **Farmer** | **Wife** | **Child** | **Farmer** | **Wife** | **Child** |
| | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** | **Pearson Corr. (p-value)** |
| **Entryway** | 0.49* (0.005) | 0.04 (0.83) | -0.40 (0.16) | 0.13 (0.45) | 0.44* (0.02) | 0.54 (0.13) |
| **Living Room** | 0.31** (0.08) | 0.007 (0.97) | -0.40 (0.17) | 0.03 (0.84) | 0.35** (0.08) | 0.72* (0.02) |
| **Master Bedroom** | 0.28 (0.12) | -0.04 (0.81) | -0.50** (0.08) | 0.09 (0.62) | 0.33** (0.09) | 0.72* (0.02) |
| **Kitchen** | 0.36* (0.04) | -0.10 (0.60) | -0.40 (0.15) | 0.11 (0.52) | 0.35** (0.07) | 0.56 (0.11) |
| **House Avg.** | 0.38* (0.03) | -0.07 (0.72) | -0.47 (0.10) | 0.09 (0.59) | 0.41* (0.04) | 0.58 (0.1) |

*p-value < 0.05 and considered statistically significant

**p-value considered borderline statistically significant (between 0.05 and < 0.10)

Pearson's Correlation coefficients between vacuum dust atrazine concentrations from the four locations of each home, the house average dust atrazine concentration and unadjusted and creatinine-adjusted total urinary atrazine concentrations of the farmers,

their spouses and children (Farmer and Family) and the spouses and children only (Family only) are presented in Tables 4.8 and 4.9.

During the non-planting season, comparing the farmer and family as a whole, and the family only, the total urinary atrazine concentration is positively correlated with vacuum dust atrazine concentrations of all the four locations and the house average (p-value < 0.05).

**Table 4.8: Association between Dust Atrazine Concentrations and Total Urine Atrazine Concentrations**

| | Planting Season | | Non-planting Season | |
|---|---|---|---|---|
| | **Farmer and Family** | **Family only** | **Farmer and Family** | **Family only** |
| | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** |
| **Entryway** | 0.18 (0.11) | -0.06 (0.71) | 0.29* (0.01) | 0.4* (0.01) |
| **Living Room** | 0.12 (0.30) | -0.92 (0.57) | 0.24** (0.05) | 0.38* (0.02) |
| **Master Bedroom** | 0.02 (0.81) | -0.18 (0.26) | 0.26* (0.03) | 0.38* (0.02) |
| **Kitchen** | 0.04 (0.71) | -0.19 (0.23) | 0.26* (0.03) | 0.35* (0.04) |
| **House Avg.** | 0.08 (0.5) | -0.19 (0.24) | 0.27* (0.02) | 0.4* (0.02) |

*p-value < 0.05 and considered statistically significant

**p-value considered borderline statistically significant (between 0.05 and < 0.10)

During the planting and non-planting season, the farmer and family creatinine-adjusted total urinary atrazine concentrations are positively correlated with the vacuum dust atrazine concentrations of the entryway, the living room (p- value < 0.05) and the house average in the non-planting season. During the non-planting season, the family only creatinine-adjusted total urinary atrazine concentrations are positively correlated

with the vacuum dust atrazine concentrations of the entryway, the living room, and the house average (p-value $\leq 0.05$).

**Table 4.9: Association between Dust Atrazine Concentrations and Creatinine-Adjusted Total Urine Atrazine Concentrations**

| | Planting Season | | Non-planting Season | |
|---|---|---|---|---|
| | **Farmer and Family** | **Family only** | **Farmer and Family** | **Family only** |
| | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** | **Pearson Corr. Coeff. (p-value)** |
| **Entryway** | 0.25* (0.03) | 0.11 (0.48) | 0.28* (0.02) | 0.37* (0.03) |
| **Living Room** | 0.27* (0.02) | 0.21 (0.18) | 0.23** (0.05) | 0.33** (0.05) |
| **Master Bedroom** | 0.06 (0.57) | -0.02 (0.90) | 0.21** (0.08) | 0.28 (0.10) |
| **Kitchen** | 0.12 (0.30) | -0.05 (0.72) | 0.22** (0.07) | 0.27 (0.11) |
| **House Avg.** | 0.15 (0.2) | -0.02 (0.89) | 0.24* (0.04) | 0.33** (0.05) |

*p-value < 0.05 considered statistically significant

**p-value considered borderline statistically significant (between 0.05 and < 0.10)

EPA acute and chronic reference doses for atrazine are described in table 4.10. These reference doses are derived from animal toxicity studies (EPA, 2002). Different routes and duration of exposure are used while determining the reference doses (RfD). The RfD takes into account an uncertainty factor of 100 to account for intra- and inter-species variability (Curwin et. al., Submitted to Environmental Health Perspectives). The Food Quality Protection Act (FQPA) mandates the evaluation of food tolerances on the basis of cumulative risk from substances sharing a common mechanism of toxicity (EPA Atrazine IRED, 2003). Biological monitoring is advantageous as it takes into account exposures from all sources and routes (Curwin et. al., Submitted to Environmental Health

Perspectives). The population adjusted dose (PAD) characterizes the dietary risk of a chemical. PAD reflects acute or chronic RfD that has been adjusted to account for the FQPA safety factor (EPA Atrazine IRED, 2003).

**Table 4.10: EPA Acute and Chronic Reference Doses for Atrazine**

| Pesticide | RfD[a] (μg/kg/day) | Study | Toxicity Endpoint |
|---|---|---|---|
| Acute reference dose | 10[b] | Developmental toxicity study in rat and rabbit | Delayed ossification in fetuses; decreased body weight gain in adults |
| Chronic reference dose | 1.8[b] | Six months luteinizing hormone (LH) surge study in rat | Attenuation of preovulatory LH surge |

[a] EPA definition of RfD: "An estimate of a daily oral exposure for an acute duration (24 hours or less) or chronic duration (up to a life time) to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime".

[b] Denotes a population adjusted reference dose (PAD) which incorporates an additional FQPA safety factor of 10.

The mean atrazine doses of farmers and their family members are presented in table 4.11. There is no significant difference between the mean doses of the planting and non-planting season for all subjects except for farmers who hired a commercial applicator for pesticide application (p-value = 0.03).

**Table 4.11: Mean Pesticide Doses (μg/kg/day) for Self-Applied and Commercially-Applied Farmers, their Spouses, and Children**

| | Self-applied Planting season | | Self-applied Non-planting season | | |
|---|---|---|---|---|---|
| **Subjects** | **Mean (SD)** | **Range** | **Mean (SD)** | **Range** | **p-value** |
| **Farmer** | 2.2 (1.78) | 0.01 – 5.1 | 1.5 (1.4) | 0.008 - 4 | 0.18 |
| **Spouse** | 1.0 (0.66) | 0.03 – 2.1 | 2.5 (3.46) | 0.01 – 11.2 | 0.2 |
| **Children** | 0.4 (0.19) | 0.01 – 0.6 | 0.3 (0.32) | 0.1 – 0.8 | 0.65 |
| | **Commercially-applied Planting season** | | **Commercially-applied Non-planting season** | | |
| **Subjects** | **Mean (SD)** | **Range** | **Mean (SD)** | **Range** | **p-value** |
| **Farmer** | 0.9 (0.89)[a] | 0.01 – 3.3 | 1.6 (1.07)[b] | 0.007 – 3.5 | 0.03 |
| **Spouse** | 1.4 (1.46) | 0.01 – 5.8 | 1.5 (0.84) | 0.05 – 2.7 | 0.8 |
| **Children** | 0.6 (0.55) | 0.003 – 1.3 | 0.2 (0.26) | 0.02 – 0.5 | 0.15 |

[a, b, c] Means with the same letter are not significantly different from each other based on p-value < 0.05 obtained from paired t-test

The highest dose estimates for self-applied farmers were 5.1 and 4 μg/kg/day in the planting and non-planting seasons respectively. The highest dose estimate for spouses was 11.2 μg/kg/day in the non-planting season, which is above the EPA acute RfD (Table 4.11). The percent of study subjects whose estimated doses exceeded the EPA reference values is shown in table 4.12. Fifty percent of farmers that self-applied their pesticides had a dose estimate that exceeded the EPA chronic population-adjusted reference value for atrazine. None of the children in the planting or non-planting seasons had atrazine dose estimates exceed the EPA reference values. This could be because of the small sample size for children among self-applied and commercially-applied farm families. This finding is similar to Curwin's findings for children for atrazine, metolachlor, and glyphosate (Curwin et. al., submitted).

**Table 4.12: Percent of Individuals with Estimated Dose exceeding Atrazine Reference Values in the Planting Season**

| Reference value | Farmer | | Spouse | | Children | |
|---|---|---|---|---|---|---|
| | Self N = 10 | Commercial N = 22 | Self N = 9 | Commercial N = 18 | Self N = 6 | Commercial N = 8 |
| NOAEL | 0 | 0 | 0 | 0 | 0 | 0 |
| Acute RfD | 0 | 0 | 0 | 0 | 0 | 0 |
| Acute PAD | 0 | 0 | 0 | 0 | 0 | 0 |
| Chronic RfD | 0 | 0 | 0 | 0 | 0 | 0 |
| Chronic PAD | 50 | 18 | 22 | 28 | 0 | 0 |

Note: NOAEL: no observable adverse effect level; RfD: reference dose; PAD: population adjusted reference dose

However, there were more farmers and their spouses whose estimated doses exceeded the EPA chronic PAD in the non-planting season compared to the planting season (Table 4.13). None of the study subjects (farmers, their spouses and children) exceeded the NOAEL and acute EPA reference doses in both the planting and non-planting seasons.

**Table 4.13: Percent of Individuals with Estimated Dose exceeding Atrazine Reference Values in the Non-Planting Season**

| Reference value | Farmer | | Spouse | | Children | |
|---|---|---|---|---|---|---|
| | Self N = 10 | Commercial N = 22 | Self N = 9 | Commercial N = 17 | Self N = 4 | Commercial N = 5 |
| NOAEL | 0 | 0 | 0 | 0 | 0 | 0 |
| Acute RfD | 0 | 0 | 0 | 0 | 0 | 0 |
| Acute PAD | 0 | 0 | 11 | 0 | 0 | 0 |
| Chronic RfD | 0 | 0 | 0 | 0 | 0 | 0 |
| Chronic PAD | 30 | 50 | 56 | 41 | 0 | 0 |

Note: NOAEL: no observable adverse effect level; RfD: reference dose; PAD: population adjusted reference dose

**Discussion**

In this study, evening and morning urine samples were collected from the farmers, their spouses, and children during both the planting and non-planting seasons. This study found that there was no statistical significant difference observed between the evening and morning total atrazine concentrations of farmers, their spouses and children, during both the planting and non-planting seasons for self-applied and commercially-applied farm homes. This trend was also observed in the creatinine-adjusted total urinary concentrations of the farmers and their families in both the seasons. This is indicative of the excretion interval of atrazine after pesticide application in the planting season. The absence of a significant decrease in urinary atrazine concentrations from evening to morning (acute change) in the non-planting season is indicative of the continuous presence of this pesticide inside farm homes irrespective of pesticide application. Similarly, the absence of a significant decrease in urinary atrazine concentrations from planting to non-planting season (chronic change) is indicative of the persistence of atrazine exposures irrespective of application time.

Estimation of correlation coefficients using parametric measures to study the correlation between vacuum dust atrazine concentrations from the four locations of each home, the house average dust atrazine concentration and unadjusted and creatinine-adjusted total urinary atrazine concentrations of the farmers and their families revealed that, among homes that hired a commercial applicator for pesticide application, the total urinary atrazine concentration in the non-planting season was marginally positively correlated with the kitchen dust atrazine concentration. Among those farm families that self-applied their pesticides, the dust atrazine concentrations for the entryway, living

room, and the house average were positively correlated with the creatinine-adjusted total urinary atrazine concentration. Curwin et al. (2005b) found significant association between wipe samples and urinary levels of atrazine. Similar positive correlations were found between these types of samples among pesticide applicators (Tuomainen et al., 2002).

Correlation coefficients among family members of those that self-applied their pesticides and those that commercially-applied during the planting and non-planting seasons showed that during the non-planting season, among those homes that hired a commercial applicator for pesticide application, the total urinary atrazine concentrations of the farmers' spouses was highly correlated with the vacuum dust concentrations in the master bedroom.

Irrespective of the type of pesticide application, during the planting season, the farmers' total urinary atrazine concentrations were strongly positively correlated with the vacuum dust concentrations in the entryway, the kitchen, and the average house dust concentration. During the non-planting season, the spouses' total urinary atrazine concentrations were positively correlated with the vacuum dust concentrations in the entryway and the average house dust concentration. Likewise in the non-planting season, the children's total urinary atrazine concentrations were positively correlated with the vacuum dust concentrations in the living room, the master bedroom, and the average house dust concentration.

Pearson's Correlation coefficients between vacuum dust atrazine concentrations from the four locations of each home, the house average dust atrazine concentrations and unadjusted and creatinine-adjusted total urinary atrazine concentrations of the farmers,

their spouses and children (farmer and family) and the spouses and children only (family only) found that the total urinary atrazine concentrations were positively correlated with vacuum dust atrazine concentrations of all four locations and the house average during the non-planting season. During both the seasons, the farmer and family creatinine-adjusted total urinary atrazine concentrations were positively correlated with vacuum dust atrazine concentrations of the entryway, the living room, and the house average. Curwin's study (2007) found that pesticide dust concentration in the study homes was positively associated with urinary metabolite concentration for all pesticides except atrazine in farm mothers, but the associations were not statistically significant. Curwin also found that in farm homes, the father's urinary pesticide metabolite concentrations were correlated with the mother's and children's concentrations. The current study results indicate that vacuum dust concentrations of some of the four locations sampled in the self-applied study homes and those of commercially-applied homes were positively correlated with the unadjusted and creatinine-adjusted total urinary atrazine levels in farmers, their spouses and children.

There was only a slight difference in comparisons between dust and the unadjusted and creatinine-adjusted urine atrazine concentrations of the farmers and their families in both the planting and non-planting season based on applicator type. The positive association of atrazine concentrations from vacuum dust samples of farm homes with the total urinary atrazine concentrations of farmers and their families from those farm homes indicates that the absorbed doses of atrazine are associated with the presence of atrazine in their homes. This is applicable not only to the planting season when atrazine is actively applied to the fields, but also to the non-planting season when there is

no atrazine application. The positive detection of atrazine in the vacuum dust samples and atrazine and its metabolites in the urine samples of almost all the study subjects in both the planting and non-planting seasons indicates that the herbicide atrazine is causing exposure to farm families inside their homes irrespective of their involvement in pesticide application and season.

The atrazine dose estimates presented here indicate the significance of atrazine exposure among farmers, their spouses, and children. While Curwin studied only children's pesticide dose estimates, this study included the farmers and their spouses dose estimates in comparison to reference values (Curwin et al., submitted). The doses were higher for farmers in the non-planting season and for their spouses in both seasons. Total urinary atrazine concentrations were higher in the non-planting season for farmer's family members which may explain why doses were similarly higher. However, a considerable number of atrazine dose estimates of farmers and their spouses were well above the EPA chronic population-adjusted reference value. Of concern, however, is the dose estimate of a self-applied farmer's spouse which is higher than the EPA acute population-adjusted reference value during the non-planting season. These results appear to contrast with those of Curwin's where none of the children's dose estimates exceeded reference values for atrazine (Curwin et al., submitted).

Fenske's study on children of agricultural workers reveled that 56% of azinphos-methyl estimated doses exceeded the EPA reference values, and 44% of those of non-agricultural children exceeded the EPA reference value (Fenske et al., 2000a, 2000b). None of these estimates exceeded the NOAEL for this compound. Acquavella et al. observed that none of their estimated doses for glyphosate exceeded the EPA chronic

reference values. In spite of these similar studies conducted in the past, the strengths of the current study are to estimate the absorbed doses of farmers and their family members; and to study the trend during both the planting and non-planting seasons. Atrazine concentrations from food residues and drinking water were not collected as part of this study and are potential limitations in assessing complete exposure of farm families to atrazine. The presence of estimated doses above the EPA chronic population-adjusted reference values in both the seasons provides an indication of the significance of pesticide persistence and exposure.

**Conclusion**

Comparison of the evening and morning urinary atrazine levels revealed that the total urinary atrazine concentrations of farmers and their families did not significantly decrease over night in both the planting and non-planting seasons for self and commercially-applied farm homes. The positive association of dust atrazine concentrations of farm homes with the total urinary atrazine concentrations of farmers and their families from those farm homes indicates that the absorbed doses of atrazine are associated with the presence of atrazine in their homes. The persistence of this triazine herbicide throughout the year inside farm homes is likely the cause of detectable atrazine levels in farm homes and the urine samples of farmers and their families long after the application season has ended. An estimation of atrazine doses from the farmers, their spouses and children allowed comparison to EPA reference values. Estimated doses being higher than the reference values in both seasons indicate the presence of this pesticide in farm homes. This persistence can cause potential exposures to not only the

farmers, but also their spouses, children and anyone living in their household. This can lead to the bioavailability of this herbicide atrazine in farm families not only during pesticide applications but also during non-application times.

# CHAPTER 5

# CONCLUSION

This study found that all homes located on a farm, whether they self-apply or commercially-apply pesticides had atrazine contamination inside the homes. The locations sampled in these homes were definitely contaminated by atrazine and are comparable to pesticide concentrations found in farm homes in the study conducted by Curwin et al. (2005a, 2005b). The overall home atrazine dust concentrations for both the self-applied and commercially-applied homes were significantly higher in the planting season compared to the non-planting season and decreased overtime. Certain locations inside the farm homes had a difference in atrazine dust concentrations between homes where they applied the pesticides themselves and where they hired a commercial applicator. Atrazine dust concentrations did not decrease significantly over a period of one year in homes that hired a commercial applicator for pesticide application for certain locations. However, the house average (all locations combined) atrazine concentrations did decrease significantly. It is the amount of atrazine applied, the number of acres sprayed, and the time spent in atrazine application that determines the presence and persistence of this herbicide in dust inside farm homes and the potential exposures that can occur in farm families both in the planting and non-planting seasons. One of the most important findings of this study is the identification of atrazine concentrations inside homes during the non-planting season when atrazine is not handled, signifying the presence of pesticides irrespective of handling times. Also, the finding that it is present continuously over time in farm homes that hire a commercial-applicator disproves the

common belief that "farm homes that do not handle this pesticide are free from its exposure" (Chapter 2).

This study also found that all study subjects in all homes located on a farm, whether they self-apply or commercially-apply had atrazine exposures causing detectable concentrations of atrazine and its metabolites in their urine samples. The whole family total urinary atrazine concentrations for both the self-applied and commercially-applied homes were not statistically significantly different between the planting and non-planting season. This is suggestive of take-home pesticide exposure as has been observed in previous studies by Curwin et al. (2005a, 2007). Urinary total atrazine concentrations did not decrease significantly over a period of one year in homes that applied their own pesticides and in those that hired a commercial applicator for pesticide application. It is the persistence of this herbicide inside farm homes to cause potential exposures not only to the farmers, but also their spouses, children, and anyone living in their household. This presence and persistence of atrazine can lead to its bioavailability in farm families both in the planting and non-planting seasons. This can be compared to the study of the use and subsequent disuse of chlorpyrifos in residential applications still causing ever-present concentrations of this pesticide in household environments (CDC, 2002; Curwin et al., 2005b; Fenske et al., 2002).

One of the most important findings of the current study is the identification of atrazine concentrations in subjects' urine during the non-planting season. It is disturbing to find that atrazine does not decrease over time. In fact, certain study participants in the non-planting season had higher urinary atrazine concentrations. This prompts the

importance and necessity to study all the other exposure pathways in future studies and attempt to fully assess exposure through all routes (Chapter 3).

This study found that all study subjects in all homes located on a farm, whether they self-apply or commercially-apply had atrazine levels detected inside their homes and in their urine in both the planting and non-planting seasons. Comparison of the evening and morning urinary atrazine levels revealed that the total urinary atrazine concentrations of farmers and their families did not significantly decrease over night in both the planting and non-planting seasons for self and commercially-applied farm homes. The positive association of dust atrazine concentrations of farm homes with the total urinary atrazine concentrations of farmers and their families indicates that the absorbed doses of atrazine are associated with the presence of atrazine in their homes (Chapter 4). This compares to previous studies by Curwin et al. (2005b) who found significant association between wipe samples and urinary levels of atrazine.

Pesticide dose estimates were calculated for all the study participants in this study. A considerable number of atrazine dose estimates of farmers and their spouses were well above the EPA chronic population-adjusted reference value. While Curwin studied only children's pesticide dose estimates, this study included the farmers and their spouses' dose estimates in comparison to reference values (Curwin et al., 2007, submitted). None of the children's dose estimates for atrazine in the current study nor Curwin's study exceeded the EPA reference values. An important finding of this study is the estimation of absorbed doses of atrazine and the number of study participants whose estimates were higher than the reference values. The presence of estimated doses above

the EPA chronic population-adjusted reference values in both seasons provides an indication of the significance of pesticide persistence and exposure (Chapter 4).

**Strengths of the Study**

Some of the strengths of this study are the results indicating that homes that did their own atrazine application are not significantly different than those that had a commercial application in terms of atrazine application, its presence, and its exposure. This finding is contrary to the common belief that farm homes that hire a commercial applicator to spray the pesticides on their fields would not be exposed to pesticides applied on the fields. This study also reveals that the whole family total urinary atrazine concentrations did not significantly decrease over a period of one year for farm homes that did their own atrazine application and those that hired a commercial applicator. The positive detection of atrazine and its metabolites in the urine samples of almost all the study subjects in both the planting and non-planting seasons indicates that the herbicide atrazine causes exposure to farm families inside their homes irrespective of their involvement in pesticide application. The estimation of atrazine absorbed doses of farmers and their family members during both the planting and non-planting seasons is one of the strengths of this study, and addresses some of the important research questions from previous studies.

**Limitations of the Study**

This study focuses on measuring atrazine for vacuum dust collected from home floors and from urine samples of the study subjects. There are limitations to the study

design. Not all exposure pathways were sampled. Food was potentially a source of pesticide exposure in farm families, but beyond asking questions about participants' gardens, no data were collected on food pesticide residues. Drinking water was known to be a potential source of exposure to pesticides in general and more specifically to atrazine. Drinking water samples were not collected and analyzed in this study. Also, atrazine levels in finished/treated drinking water at the water treatment plants that supply water to the study subjects have not been sampled and analyzed for atrazine levels. This can be a potential source of atrazine exposure if the water consumed by the study subjects is contaminated with atrazine whether the source is treated water supply or from their own water wells. Similarly, dermal exposure could be a significant route of exposure, but no effort was made to quantify dermal deposition and absorption in this study.

The relatively small sample size for children is another limitation of this study. Study subject recruitment is a challenging task and participation rates are low for most farm research studies. It is difficult to find farm families with children at home to participate in such studies, as most of them would be in school or away from home during the school year. This prompts every effort that can be made to identify and implement incentives and resources to increase study participation in future research studies.

**Recommendations**

The findings presented in this study identified the persistence of pesticides in farm homes and pesticide exposure among farm families during both planting and non-planting seasons. The results of this study will be used to develop more comprehensive

sampling and survey methods for measuring the total bioavailability of pesticides in a larger study which will ultimately lead to interventions and recommendations for reducing long-term pesticide exposures to farmers in their homes. The research methods used in this study will provide guidance towards developing a larger study to evaluate the relationship between the sources of pesticide contamination, migration into homes, and potential exposure to farmers and their families. This will be valuable to help design a larger study leading to the characterization of the total bioavailability of pesticides, and developing recommendations for the prevention of pesticide exposure to farmers' families. All study subjects would be provided the results of the dust and urine samples and an overall summary report. Results of this study will be disseminated in the scientific literature and in educational materials directed to farm families to make them more aware of the environmental presence and persistence of pesticides and potential exposures and will provide practical recommendations for prevention. It is hoped that these results will lead to the development and testing of interventions that can reduce pesticide contamination in the homes of farm families and would ultimately reduce and/or eliminate their exposures to these chemical substances. This could lead to the prevention of unwanted health outcomes and preservation of agricultural health in farming communities, as part of a national and global public health initiative to protect people's health.

APPENDIX A

FARM FAMILY PESTICIDE EXPOSURE STUDY

PARTICIPANT INTERVIEW

**Parental or Guardian Information**

(To be asked to each parent or guardian)

Name: _____

Home ID #: _____

Subject ID #: _____

Date: _____

(Month/Day/Year)

Interviewer: _____

What is your date of birth? _ _ _ _ _ _ Age_____

(Month/Day/Year)

What is your height?_____ Weight?_____

Interviewer: Please note the sex of the interviewee:

Male _____

Female _____

What is your current job? _____

Do you carry out any farm related work? Yes_____ No_____

(i.e. any work directly involved in farm production)

If so, please describe _____

_____

_____

Do you carry out any work that involves handling pesticides? Yes_____ No_____

If so, please describe _____

_____

_____

_____

_____

Some of the questions we will be asking you relate to the operation of the farm, particularly the application of pesticides, while others relate to care of the home and the children. In order to minimize the burden of asking questions to both members in the family, we would like you to designate a primary respondent for each of these areas. This of course should be the person who you feel is most knowledgeable.

Farming and pesticide application: Myself_____ Spouse:_____

Home and child care: Myself_____ Spouse:_____

**Information About Children**

How many children are living in your home?_____

List their names and ages:

| Name | Age | Sex | Birth date | Weight (Lbs) | Height (Ft, In) |
|---|---|---|---|---|---|
| | | | | | |

(To be filled out for each child participating in the study).

Name of child: _____

Subject ID #: _____

Child's date of birth? __ __ __ __ __ __ Age_____

(Month/Day/Year)

Child's sex: Male _____ Female _____

On a typical spring or summer day, how many hours does the child spend:

Indoors?_____ Outdoors?_____

When indoors, where does he/she play most often?_____

Bedroom? _____

Living/family room? _____

Kitchen? _____

Laundry area? _____

Play room (if any)? _____

Other? _____

How much time does he/she spend in the:

Bedroom? _____

Living/family room? _____

Kitchen? _____

Laundry area? _____

Play room (if any)? _____

Other? _____

When outdoors, where does he/she play most often?_____

How many hours are spent there? _____

Does the child play in the crop fields? Yes_____ No_____

Is the child involved in any farm chores? Yes_____ No_____

If so, please describe:_____

_____

_____

_____

_____

Does the child handle or apply pesticides? Yes_____ No_____

If so, what does your child wear when handling pesticides?_____

_____

_____

If so, where does your child change out of work clothes? _____

_____

_____

Does the child live with you all of the time? Yes_____ No_____

If not, how many days since pesticide applications began has he/she resided elsewhere?

_____

Does your child go to school? Yes_____ No_____

If yes, how many hours/day? _____

How many days per week? _____

Is school still in session? Yes_____ No_____

If no, when did the school year end? _____

If yes, how many days has your child been at school since pesticide applications began? _____

Does your child attend daycare or go to a private home for childcare on a regular basis?

Yes_____ No_____

If yes, how many hours per day?_____

How many days per week?_____

Is it a private residence or commercial daycare facility? _____

Has he/she done this since pesticide applications began? Yes_____ No_____

If yes, how many days has your child been at daycare since pesticide applications began? _____

**Household Information**

(To be asked to the parents or guardians)

What year did you move into this home? _____

What year was this home constructed? _____

Do you own or rent this house? _____

Has this home been sprayed inside with insecticides in the last month:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes, which insecticide(s) were used? _____

_____ _____

_____ _____

_____ _____

Has this home been sprayed inside with insecticides in the last 12 months:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes which insecticide(s) were used? _____

_____ _____

_____ _____

_____ _____

Has your lawn been treated with pesticides (insecticides, herbicides, or fungicides) in the last month:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes which pesticide(s) were used? _____

_____ _____

_____ _____

_____ _____

Has your lawn been treated with pesticides (insecticides , herbicides, or fungicides) in the last 12 months:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes which pesticide(s) were used? _____

_____ _____

_____ _____

_____ _____

Do you have a garden? Yes____ No_____

If yes, has your garden been sprayed with pesticides (insecticides, herbicides, or fungicides) in the last month:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes which pesticide(s) were used _____

_____ _____

_____ _____

_____ _____

Has your garden been sprayed with pesticides (insecticides, herbicides, or fungicides) in the last 12 months:

Professionally? Yes ____ No ____ Don't know ____

Personally? Yes ____ No ____ Don't know ____

If yes which pesticide(s) were used? _____

_____ _____

_____ _____

_____ _____

Do you consume food from the garden? Yes_____ No_____

If yes, have you consumed food from the garden in the last month?

Yes___ No___

How often do you vacuum your carpet?

Less than once a month _____

Once a month _____

Twice a month _____

Once a week _____

More than once a week _____

How often do you mop or vacuum your floors?

Less than once a month _____

Once a month _____

Twice a month _____

Once a week _____

More than once a week _____

Are the work clothes of the farm worker laundered separately from the rest of your family's clothes? Yes ____ No ____

Do you have a dog? Yes_____ No_____

If yes, does your dog spend time both indoors and outdoors? Yes____ No____

Do your children play with the dog? Yes_____ No_____

Do you have a cat? Yes_____ No_____

If yes, does your cat spend time both indoors and outdoors? Yes____ No____

Do your children play with the cat? Yes_____ No_____

Do you have a door mat for your outside doors? Yes_____ No_____

If yes, record which doors:

Front door _____

Back door _____

Garage door _____

Other door (specify) _____

**Crop and Pesticide Application Information**

(To be asked to the person living in the farm household who is the principal farmer)

Please fill in the table for pesticides recently sprayed

What crop(s) are you currently growing? _____

or will be growing this season? _____

_____

How close are the fields (in feet) to the house? _____

How many acres is each crop? Crop:_____ Acres:_____

Crop: _____ Acres: _____

Crop: _____ Acres: _____

Crop: _____ Acres: _____

Over how many days did pesticide (herbicide, insecticides, fungicide) spraying occur for each crop?

Crop: _____ Days: _____

Crop: _____ Days: _____

Crop: _____ Days: _____

Crop: _____ Days: _____

On average, how many hours each day were spent mixing, loading, and applying pesticides to each crop?

Crop: _____ Hours: _____

Crop: _____ Hours: _____

Crop: _____ Hours: _____

Crop: _____ Hours: _____

Who mixed and loaded the pesticide(s)?

Yourself _____

Custom applicator _____

Other farm worker _____

Who applied the pesticide(s)?

Yourself _____

Custom applicator _____

Other farm worker _____

Where did the mixing, loading, and spraying take place? _____

How many acres in total were sprayed with pesticides? _____

What kind of application equipment is used to spray your fields?

Aerial _____

Groundboom _____

Other? If other please describe:_____

If you apply your own pesticides:

Where are your pesticides stored?_____

Do you have a closed (ie. completely closed with air conditioning) or open cab?

What protective clothing do you wear when mixing, loading, and applying pesticides:

Gloves? _____

Long pants? _____

Long shirt? _____

Respirator? _____

Chemical protective clothing (eg. tyvek, PVC)? _____

Rubber boots? _____

Goggles? _____

In addition to your own crops, do you spray other people's crops? Yes____ No____

If yes, on average how many acres do you spray in a season?_____

Do you own or have livestock on your farm? Yes_____ No_____

If yes, what livestock do you own or have?_____

_____ _____

_____ _____

Do you apply insecticides to the livestock? Yes____ No____

If yes, which insecticides are used and how much:

Livestock: _____ Pesticide: _____ Pounds: _____

Livestock: _____ Pesticide: _____ Pounds: _____

Livestock: _____ Pesticide: _____ Pounds: _____

Where do you change out of your work clothes?

_____

If you change outside the home, do you bring your work clothes in the home?

Yes_____ No_____

Where do you change out your work shoes?_____

If you change your work shoes outside the home, do you bring your work shoes in the home? Yes_____ No_____

How do you travel from your work site (fields) to home? _____

Is the car/truck driven to/from work site and home the same car/truck used for family transportation? Yes_____ No_____

Do your kids spend any time in this vehicle? Yes_____ No_____

If yes, approximately how many hours/week would they spend in this vehicle? _____

**Pesticides recently sprayed (Planting Season)**

Address:_____ Home ID #_____ Date:_____

| Pesticide Name (EPA Registration #) | Active ingredient | Date Sprayed | Time Sprayed (duration) | Application rate | Amount Sprayed (wt or vol) | Who mixed loaded and applied? (Farmer, custom applicator) | What application equipment was used? | Acres Sprayed (Crop) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

# APPENDIX B

## NON-PLANTING SEASON QUESTIONNAIRE

1. When was the last pesticide application done?

   - Date _____
   - Pesticides applied _____

     _____

     _____
   - Amount of Pesticides applied

     Pesticide Quantity

     1.

     2.

     3.

     4.

     5.

     6.

2. Were any pesticides used since the end of spraying and planting?

   If yes, list them: 1.

   2.

   3.

   4.

   5.

   6.

3. Were any of these pesticides applied by you/ custom applicator?

   Circle one:

   1. Farmer
   2. Custom applicator
   3. Both

4. When was the last time that you/custom applicator applied pesticides after the end of the planting season? _____(Date)

**Pesticides recently sprayed (Since last visit)**

Address:_____ Home ID #_____ Date:_____

| Pesticide Name (EPA Registration #) | Active ingredient | Date Sprayed | Time Sprayed (duration) | Application rate | Amount Sprayed (wt or vol) | Who mixed loaded and applied? (Farmer, custom applicator) | What application equipment was used? | Acres Sprayed (Crop) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
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# APPENDIX C

# LABORATORY ANALYSIS OF PESTICIDES FOR THE IOWA FARM FAMILY STUDY

by

Marcia Nishioka, Task Leader

Charles Knott, Project Director

BATTELLE

Centers for Public Health Research and Evaluation

## Analytical Method for Analysis of Atrazine in Dust

The dust samples were sieved to 150 μm using a 100 mesh stainless steel sieve. The fine dust was weighed to two significant figures after the decimal (e.g., 4.76 g) and returned to the original sample bottle. The coarse dust, which generally consisted of pet hair- carpet filter, and large particles, was discarded. A 0.5 g aliquot of each fine dust sample was weighed out into a centrifuge tube and spiked with 250 ng of the surrogate recovery standard (SRS) $^{13}C_3$-atrazine. For samples with less than 0.5 g of dust, a smaller quantity of dust (either 0.2 or 0.1 g) was weighed out and spiked with a proportionally smaller quantity of the SRS. A 12 mL volume of 1:1 (v:v) hexane:acetone was added; the dust was sonicated for 10 min (Branson Sonicator 5210) for extraction and then centrifuged for 10 min at 3000 rpm (Forma Scientific) to settle the dust pellet. From the 12 mL extract, 10 mL was drawn off to a Kuderna-Danish (KD) tube and concentrated to 1 mL. An additional aliquot of 5 mL of hexane was added, and the extract was reconcentrated to 1 mL.

A 1000 mg silica SPE cartridge (BakerBond, JT Baker) was conditioned in sequence with 20% acetone in ethyl acetate, dichloromethane (DCM), 15% ethyl ether in hexane, and hexane. The sample extract was added to the cartridge with hexane rinses. The cartridge was eluted in sequence with 3 mL hexane, 2 x 6 mL of 15% ethyl ether in hexane, 6 mL of DCM, and 3 x 6 mL of 20% acetone in ethyl acetate. The first three eluants were discarded, and the acetone in ethyl acetate elutions was collected as one fraction. This eluant was concentrated to 1 mL using KD concentration. For samples with proportionally smaller quantities of dust extracted, the final solvent volume was adjusted to a proportionally lower amount. The internal standard (IS) for quantification, dibromobiphenyl, was added to the extract to give a final concentration of 100 ng/mL.

Each sample batch included 10 field samples, one field sample analyzed in duplicate, a field sample spiked with either 250 ng or 2500 ng of atrazine, and a solvent method blank fortified with only the SRS. A spike check sample was also prepared, which consisted of 1 mL of the final solvent plus the quantity of the atrazine used for the fortified dust sample of that set and 250 ng of the SRS.

For the field blank samples, 250 ng of the SRS and 12 mL of extraction solvent were added directly to the sample bottle. The bottle was shaken for a minute and 10 mL was removed and processed as a field sample. Seven calibration standards, including a zero level standard, were prepared with atrazine, the SRS and the IS in ethyl acetate. The atrazine level spanned the range of 2.5-1000 ng/mL, and the SRS level spanned the range of expected concentrations.

The extracts and standards were analyzed using gas chromatography/mass spectrometry in the multiple ion detection mode (GC/MS/MID) with an HP 6890 GC

interfaced to an HP 5973 MSD at a NIOSH accredited laboratory. The samples were analyzed in a run order with samples interspersed between standards. The internal standard method of quantification was used, with a linear regression calibration curve established for atrazine and the SRS. For samples where the atrazine exceeded the calibration range, the sample extract was diluted, respiked with IS, and reanalyzed. The recovery of the SRS is reported for each sample, as well as the SRS-corrected concentration of atrazine in the dust.

The GC/MS conditions for analysis are listed in Table 1.

**Table 1: GC and MS Parameters for Analysis of Atrazine**

| **Parameter** | **Condition** | | |
|---|---|---|---|
| GC column | Phenomenex ZB-35, 30 m x 0.25 mm id; 0.25 μm film thickness | | |
| GC carrier gas | Helium at 1 ml/min | | |
| GC injector | 280 °C | | |
| GC temperature program | 70 °C (0.5 min) – 160 °C @ 30 °/min; 160 – 280 °C @ 6 °C/min; hold 280 °C 5 min; 30 min run | | |
| MID ions and retention time | **Analyte** | **MID ions** | **Retention time** |
| | Atrazine | m/z 215, 217, 200 | 10.8 min |
| | $^{13}C_3$-atrazine (SRS) | m/z 203, 218 | 10.8 min |
| | Dibromobiphenyl (IS) | m/z 312, 314 | 14.6 min |

For samples with atrazine concentrations greater than 1000 ng/mL in the analyzed solution, higher mass isotopic ions of atrazine which were not detected at lower concentration levels were at a sufficient intensity level to interfere with the monitored

quantification ion for $^{13}C_3$-atrazine. For this reason, an accurate measure of SRS recovery for samples with atrazine levels >2000 ng/g could not be obtained. For such samples, the average SRS recovery from other samples in that set that did not have detectable overlap from native isotopes was determined and this recovery value was applied.

**QC Results for Atrazine in Dust**

The recovery values for atrazine and $^{13}C_3$-atrazine in fortified dust samples are given in Table 2 for the four different spike levels tested. The spikes of 5 and 25 ng were made into a reference dust that has extremely low native atrazine levels. The spikes of 250 and 2500 ng were made into NIOSH farm dusts, chosen at random.

**Table 2: Recovery and Precision for Analysis of Atrazine in Dust**

| | | Recovery, % ± std. dev. | | |
|---|---|---|---|---|
| **Atrazine/ $^{13}C_3$-atrazine spike level** | **N** | **Atrazine** | **$^{13}C_3$-atrazine (SRS)** | **SRS-corrected atrazine** |
| 5/250 ng | 3 | 93 ± 8 | 77 ± 10 | 122 ± 14 |
| 25/250 ng | 3 | 65 ± 3 | 75 ± 2 | 86 ± 3 |
| 250/250 ng | 6 | 88 ± 8 | 90 ± 6 | 97 ± 4 |
| 2500/250 ng | 6 | 86 ± 6 | 86 ± 9 | 103 ± 11 |
| | **Average** | 83 | 82 | 102 |

The recovery of $^{13}C_3$-atrazine in the field samples averaged 86 ± 11%. The levels of atrazine in the solvent method blanks and the field blanks, and recovery of the $^{13}C_3$-atrazine in these samples, are shown in Table 3. As shown there, there was a measurable

amount of atrazine in each solvent method blank. However, this is not due to laboratory contamination. Rather, the $^{13}C_3$-atrazine is listed as 99% pure, and this small amount in the blanks is due to the residual amount of atrazine in the $^{13}C_3$-atrazine obtained from the supplier. Our estimate is that there is about 1.7% native atrazine in the labeled atrazine. This contribution of atrazine from the SRS was subtracted from each sample before calculation of the ng/g level of atrazine in the field samples or percent recovery of atrazine in fortified dust samples.

**Table 3: Level of Atrazine in Laboratory and Field Blank Samples**

| | | Concentration or Recovery, ave ± std. dev. | | |
|---|---|---|---|---|
| **Sample type** | **N** | **Atrazine conc. in extract, ng/mL** | **Equivalent Atrazine conc. in dust, ng/g** | **$^{13}C_3$-atrazine recovery, %** |
| Solvent method blank | 14 | 4.30 ± 0.83 | 8.6 ± 1.7 | 91 ± 9 |
| Field blank | 12 | 4.85 ± 1.32 | 9.7 ± 2.6 | 90 ± 9 |

The results for the analyses of the spike check solutions, given in Table 4, show excellent control over the spiking used for QC samples.

**Table 4: Recovery of Atrazine in Spike Check Samples**

| | | Recovery, % ± std. dev. | | |
|---|---|---|---|---|
| **Atrazine/ $^{13}C_3$-atrazine spike level** | **N** | **Atrazine** | **$^{13}C_3$-atrazine (SRS)** | **SRS-corrected atrazine** |
| 250/250 ng | 7 | 96 ± 13 | 97 ± 12 | 98 ± 2 |
| 2500/250 ng | 6 | 98 ± 5 | 86 ± 11[a] | 115 ± 13 |

**Limit of Quantification (LOQ) and Limit of Detection (LOD)**

The LOQ is defined operationally as the lowest concentration at which there is 10:1 S:N (signal-to-noise) ratio for the quantification ion. The LOD is the value at which there is a 3:1 S:N ratio for the quantification ion. These values for this atrazine analysis are 5ng/g for the LOQ in dust and 2.5 ng/g for LOD in dust.

Atrazine was present in all samples at levels well above the Limit of Detection (LOD) and Limit of Quantification (LOQ). The concentrations of atrazine ranged from 3-593,111 ng/g in the field samples.

# APPENDIX D

## QUANTITATION OF URINARY ATRAZINE AND ITS METABOLITES BY ON-LINE SOLID PHASE EXTRACTION-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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**Analytical Method for Analysis of Atrazine in Urine**

Chemicals

All solvents used were of analytical grade. Methanol was purchased from Tedia Company Inc. (Fairfield, OH, USA). The formic acid was purchased from Fisher Scientific (Phillipsburg, NJ, USA). Deionized water was organically and biologically purified with a NANOpure® Infinity UF from Barnstead International (Dubuque, IA, USA). Nitrogen and Argon were purchased from Airgas Inc. (Radnor, PA, USA) and had a minimum purity of 99.999%.

The native standards of ATZ and DACT were purchased from Chem Services (West Chester, PA, USA). ATZ-OH, DIA, and DEA were purchased from Dr. Ehrenstorfe GmbH (Augsburg, Germany). ATZM, DEAMM and all isotopically labeled standards were obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

Preparation of Standards Solution and Quality Control Materials

Nine working standard spiking solution, containing ATZ-OH, DACT, DIA, DEA, and DEAMM, were prepared by serial dilution with methanol of the initial stock solutions to cover the concentration range of 0.5-200 ng/mL equivalent in urine, whereas the concentration range for ATZ and ATZMM was 25-80 ng/mL. The isotope-labeled standard spiking solution was also prepared in methanol, given an approximate concentration of the individual labeled compounds of12.5 ng/mL. All standard stock solution and spiking solutions were dispensed into amber vials and stored at -5 °C until used. To prepare a calibration curve, 20 μL of each standard solution and isotope-labeled standard was added to each 1 mL of blank urine diluted with 0.1% formic acid.

Quality control (QC) materials were prepared from urine collected from multiple anonymous donors. Urine samples were combined, mixed overnight at 20°C, and then pressure filtered with a 0.45 μm filter capsule (Whatman Inc, Florham Park, NJ, USA). The urine pool was divided into four pools. Three pools were spiked with the target analytes to yield approximate concentrations of 50 ng/mL (QCH), 10 ng/mL (QCM) and 5 ng/mL (QCL), respectively. The last pool of urine was left as blank urine. This pool was used as matrix material for calibration standards and blanks. All quality control materials including blank urine pool were placed in a freezer at -20 $^{0}$C until use.

On-line Solid Phase Extraction-High Performance Liquid Chromatography(SPE-HPLC)

The On-line SPE-HPLC was performed on an Agilent 1100 system (Agilent Tech., Waldbronn, Germany) consisting of two quaternary pump, two degassers, an auto sampler with a 900-μL injection loop, and a temperature-stable column compartment. Two external 10-port switching values (Agilent Tech., Waldbronn, Germany) were inserted to facilitate the column switching performance. All the HPLC modules were programmed and controlled using the ChemStation B. 01.03 software (Agilent Tech., Waldbronn, Germany). The SPE column was a polymeric Strata-X (20x2.0 mm, 25 μm particle size, 60-$^{0}$A pore size, Phenomenex, Torrance, CA, USA) and the HPLC column was Gemini C6-Phenyl (150x4.6 mm, 3 μm particle size, 110-$^{0}$A pore size, Phenomenex, Torrance, CA, USA).

Sample Preparation and Injection

All unknown, blank, and QC samples were prepared in 1.50 mL round bottomed amber autosampler vials (Agilent Tech., Waldbronn, Germany). A 200 μL aliquot of urine was mixed with 20 μL of internal standard (equivalent to 12.5 ng/mL in urine) and homogenized. Formic acid (0.1 % in water) was added to the sample to bring the volume to 1 mL. The total injection volume was 500 μL.

Mass Spectrometry Operating Conditions

Mass spectrometry analysis was performed on a TSQ Quantum Ultra mass spectrometer (Thermo Electron, San Jose, CA) equipped with an atmospheric pressure chemical ionization (APCI) interface to generate gas phase ions of the target analytes. The mass spectrometer was programmed and controlled using Xcalibur software (Thermo Electron, FL, USA). The APCI was set in the positive mode with the following settings: 4.5 μA corona discharge current, 350 $^{0}$C vaporizer temperature, 15 psi sheath gas ($N_2$), 5 arbitrary units auxiliary gas (Ar), and 260 $^{0}$C capillary temperature.

In order to establish the appropriate multiple reaction monitoring (MRM) conditions for the individual compounds, solution of standards (500 ng/mL in MeOH) were infused into mass spectrometer. The APCI interface was optimized for the intensity of each protonated molecular species $(M+H)^{+}$. Collision-induced dissociation (CID) of each protonated molecule was performed using a collision gas (Ar) pressure of 1.5 mTorr. A total of four precursor/product ion pairs were primarily selected for further optimization in both HPLC and MS/MS quantitative analysis. Ultimately, the best precursor/product ion pair was chosen for the quantification ion and the two next best for

confirmation ions (Table 2). Each labeled internal standard was monitored using one precursor/product ion pair.

**Method Validation**

Limits of Detection

The LODs were calculated as three times the standard deviation of the noise at zero concentration ($S_0$) [Taylor, 1987]. The estimate of the noise is based on the variation in precision at concentrations close to the LODs. This was done using the four lowest calibration standards from seven available validation and analytical runs. The limit of quantification (LOQ) was defined as the lowest standard giving a consistent signal to noise (S/N) ratio of 3.

Extraction efficiency

The extraction recovery of the method was determined at two concentrations for each analyte: 5 ng/mL and 25 ng/mL for all target analytes accept ATZ and ATZMM which were 2 ng/mL and 10 ng/mL. The recoveries of compounds were calculated on the basis of the following experiments: The first experiment was representative of 100% recovery. 200 μL of urine was dispensed into the vial and mixed with 800 μL of 0.1% formic acid. Only 500 μL of sample volume was injected on the SPE column. Right before the SPE was black flushed for HPLC separation to occur, 40 μL of a solution containing both native and internal standards was injected into HPLC gradient flow (using a second Agilent 1100 autosampler). All native and isotope-labeled compounds were separated as normal and were analyzed by MS/MS. A response factor (RFa) for each analyte was calculated as the ratio of peak areas of native compound to its

corresponding labeled analogue. The second experiment tested the exact amount recovered from the SPE column. 200 μL of urine was mixed with 20 μL of native standard solution and 780 μL of 0.1% formic acid. The same amount of sample volume was injected onto SPE. Response factor (RFb) for each analyte was calculated as the ratio of peak areas of native compound to its peak areas of corresponding labeled analog from the first experiment. The two experiments differed in that for the first experiment, the native standard did not go through the SPE clean up, but did for the second (RFb). The internal standard did not go through the SPE at all and served to normalize any instrument variation. The SPE recovery was calculated as RFa/RFb. Any matrix effects were the same in both experiments.

Precision and Accuracy

The precision of the method was determined by calculating the relative standard deviation (RSD) of repeat measurements of samples from the quality control pools (QCH, QCM and QCL). Five samples from each of the QC materials were prepared and analyzed every day during a ten-day period, and the results were used to determine the within-day precision and among-day precision. Accuracy was assessed by five replicate analyses of urine spiked at four different concentrations and expressed as the percentage of the expected value.

Analyte Stability

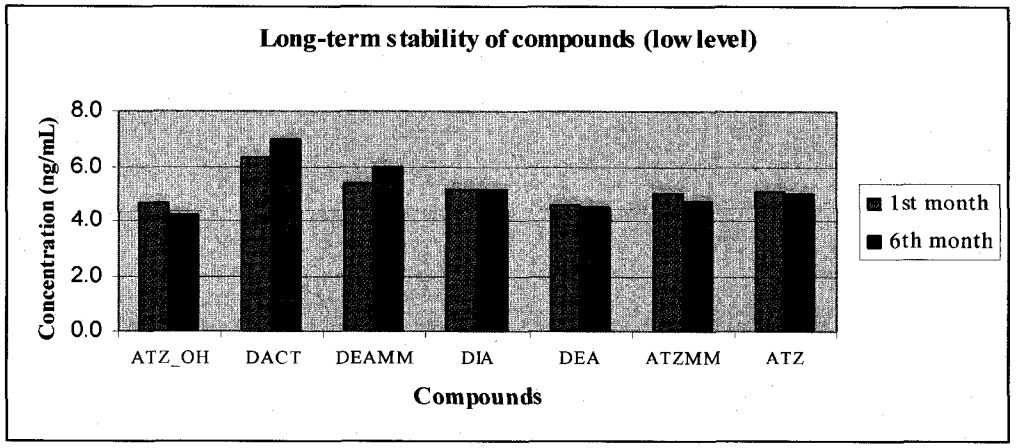
Long-term chemical stability was also tested by spiking blank urine sample with three different known amounts of standard solution and then stored at -70 $^{0}$C. For the six

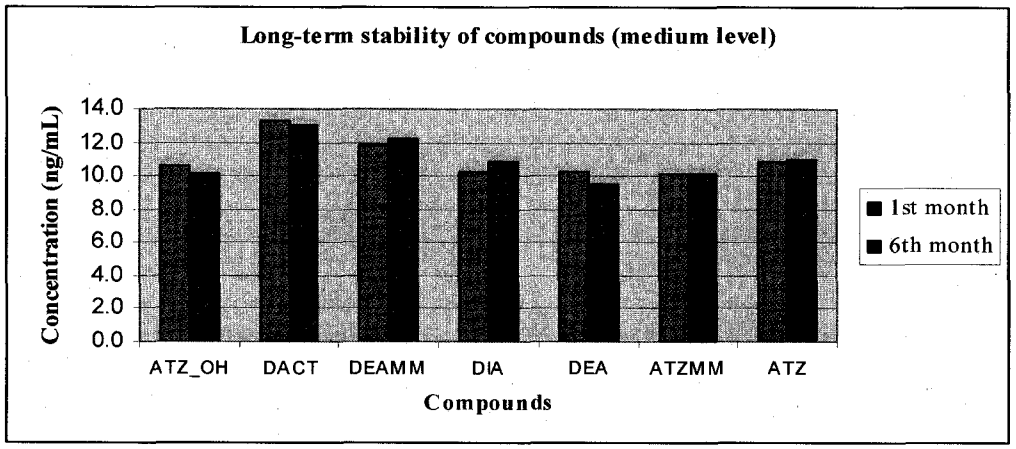
month period, the samples were removed from the storage and allowed to come to room temperature. Once samples were prepared according to previous description, the final concentrations of target analytes were approximately 5 ng/mL, 25 ng/mL, and 50 ng/mL respectively for each of five replicates.

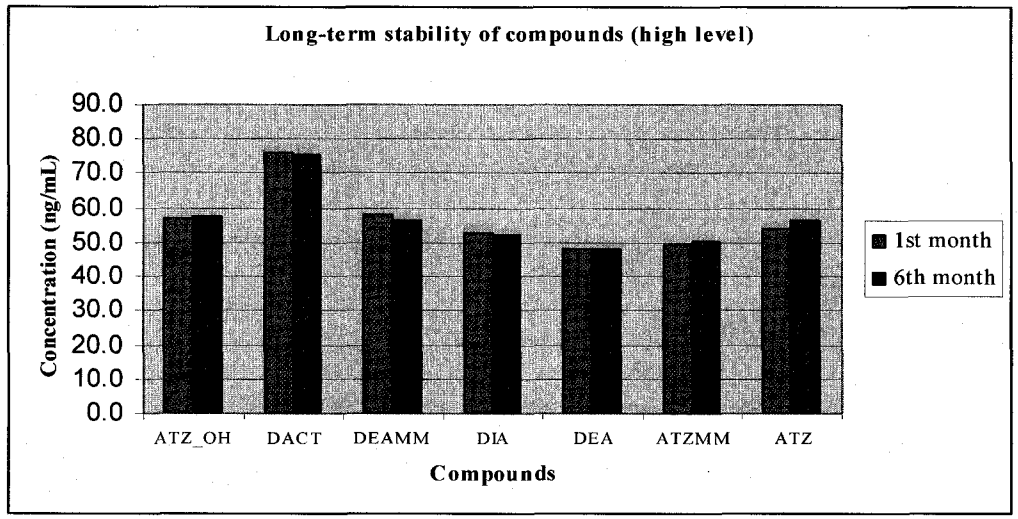
**Optimization of HPLC-MS/MS**

The liquid chromatography was optimized in order to achieve best possible separation and retention for all analytes in the same chromatographic system. The separation was performed under multi-segment gradient conditions to separate compounds that have close retention properties, as well as to force the later eluting analytes such as ATZ and ATZM come into reasonable retention time which thereby reduce the total run time. Another benefit of using multi-segment gradient conditions was to reduce cross-interference of compounds that might produce the same fragment ions because the analytes all contain the same core triazine ring and have similar chemical composition (Cai et al., 2004). This application allowed the structurally similarly compounds to be analyzed with MS/MS in different segments. The chromatographic separation of each analyte is shown in Figure 1. Interferences were observed that were not baseline separated, for the early eluting compounds particular ATZ-OH and DACT. This suggests that the methanol content in isocratic conditions plays a significant role for the chromatographic separation of these two compounds. Because of this problem, the LOD values of ATZ-OH and DACT were elevated.

**Figure 1: Long-term Stability of Target Analytes in Urine Stored at 70 $^0$C**



Based on the characterized precursor/product ion pairs of each compound, a total of three product ions, one quantitative (Q) and two confirmation ions (C1, C2) were selected (Table 1). Isotopically labeled analogues of each analyte, except for ATZ-OH, served as the internal standard for that analyte. For ATZ-OH, the only analyte for which we had no isotopically labeled compound, we used isotopic labeled analog of ATZ as the internal standard.

**Table 1: Characterized Precursor/Product Ion Pairs, the Optimum Collision Offset Energy (CE), and the Retention Time (RT) for the Native Compounds and Corresponding Isotopically Labeled Analogs**

| Compound | Precursor Ion [M+1] | Product Ions | | | Collision Energy (V) (Q, C1, C2) | RT (min.) |
|---|---|---|---|---|---|---|
| | | Q Ion | C1 Ion | C2 Ion | | |
| Native | | | | | | |
| ATZ-OH | 198 | 86 | 156 | 69 | 23, 16, 35 | 13.0 |
| DACT | 146 | 79 | 68 | 62 | 15, 26, 37 | 13.0 |
| DEAMM | 315 | 185 | 144 | 102 | 12, 23, 37 | 15.6 |
| DIA | 174 | 68 | 132 | 104 | 31, 17, 31 | 16.1 |
| DEA | 188 | 146 | 104 | 110 | 21, 25, 22 | 19.0 |
| ATZMM | 343 | 214 | 102 | 172 | 21, 39, 27 | 21.1 |
| ATZ | 216 | 174 | 104 | 68 | 18, 24, 52 | 23.5 |
| Labeled | | | | | | |
| DACT (d3) | 149 | 113 | - | - | 20 | 13.0 |
| DEAMM (Cysteine-13C3; 15N) | 319 | 186 | - | - | 20 | 15.6 |
| DIA (d5) | 179 | 137 | - | - | 21 | 16.1 |
| DEA (d6) | 194 | 144 | - | - | 21 | 19.0 |
| ATZMM (Ring-13C3) | 346 | 217 | - | - | 19 | 21.1 |
| ATZ (d5) | 221 | 179 | - | - | 19 | 23.5 |

Note: Q = Quantitative ion, C = Confirmative ion-, RT=retention time

The criteria for selecting quantitative ions included peak intensity and ion specificity as well as potential interferences from matrix. Consideration was given primarily to the most abundant ions to achieve the best sensitivity for all native

compounds including their isotopic analogs. However for native DACT and ATZ-OH, their most abundant product ions were not baseline separated with some existing interference; thus the second most abundant product ions which were almost baseline separated with the interference were then used.

In order to increase the sensitivity during MS/MS acquisition, six segments of MRM were created to detect compounds that eluted from the analytical column at different times. The first and the sixth segment were set to detect only an improbable precursor/product ion transition (200→100 @ 26) because the divert valve was set to send the mobile phase to waste.

**Recovery of On-line Solid Phase Extraction**

An attempt to extract ATZ and its metabolites from water and urine samples using solid phase extraction can be seen in various studies. Since metabolites of ATZ vary by their polarity, two types of solid phase extraction techniques were recommended. Reversed phase extraction was used for ATZ, ATZ-OH and most other chlorotriazines, while monodealkylated hydroxytriazines were extracted using strong cation exchange phase (SCX) (Lerch and Donald, 1994; Pitchon et al., 1995; Cai et al., 1995; Loos and Niessner, 1999; Ma et al., 2005; Cai et al., 2004; Li et al., 2006; Ross et al., 2006). The entire list of target analytes is in the group in which reverse phase extraction was recommended. Thus, the extraction of target compounds was evaluated using various types of commercially available reverse phase cartridges. Primary data suggested that most of the compounds were well retained in both Strata-X (Phenomenex, Torrance, CA, USA) and Bakerbond C18 (Mallinckrodt Baker, Inc. Phillipsburg, NJ, USA). However,

because DACT was retained better on a Strata-X cartridge (data not show), we chose this column for our on-line SPE. The recoveries are shown in Table 2.

Good SPE recoveries were achieved for most of the compounds (>80%) except for DACT. The increased polarity of DACT as compared to our other target analytes probably resulted in decreased sorption time resulting in lower recovery. These data also suggest that alternative SPE sorbents, particularly SCX, might provide an alternative for extraction of DACT from urine samples.

**Method Performance and Quality Control**

Both the LODs and the LOQs of DEAM, DIA, DEA, ATZM and ATZ were below 1 ng/mL which was adequate for analysis of biological samples resulted from environmental exposure (Table 2). Although, LOD of ATZ-OH was below 1 ng/mL, the LOQ was 2.5 ng/mL largely due to an insufficient chromatographic separation from a closely eluting interference. The elevated LODs and LOQs for DACT were probably a result of poorer sensitivity, low recovery from SPE, and a closely eluting interference that was not baseline separated. Regardless, the LODs and LOQs were sufficiently low to detect levels in urine samples from individuals with no known exposure.

The accuracy and precision data are summarized in Tables 2 and 3. All accuracies (also called relative recoveries) ranged from of 86-112% for all analytes, including ATZ-OH for which its isotopic analog was not available. These RSDs ranged from 4-20.5%. The RSDs were below 15% for most of the analytes. As generally expected, the RSDs were lower for higher analyte concentrations. In addition, an endogenous level of DEAM

in the urine used to calculate precision likely added to the imprecision of our calculation at the lower levels.

**Table 2: On-line SPE Recovery, Method Accuracy and Detection Limits (LODs) of Target Analytes**

| Analyte | (%) SPE recovery [a] Mean±SD | | Accuracy Mean±SD (% of expected level) | | | | LOD ng/mL | LOQ ng/mL |
|---|---|---|---|---|---|---|---|---|
| | **5 ng/mL** | **25 ng/mL** | **5 ng/mL** | **25 ng/mL** | **50 ng/mL** | **100 ng/mL** | | |
| ATZ-OH | 94±13 | 92 ± 6 | 5.0±0.4 (100.8) | 26.5±1.6 (105.8) | 47.3±1.6 (94.5) | 96.5±8.9 (96.5) | 0.2 | 2.5 |
| DACT | 69±8 | 67±10 | 5.6±0.5 (112.7) | 25.1±1.2 (100.4) | 47.8±1.7 (95.7) | 94.1±6.4 (94.1) | 2.8 | 2.5 |
| DEAMM | 100±14 | 103±16 | 4.3±0.4 (86.6) | 25.3±1.3 (101.1) | 48.3±2.0 (96.7) | 98.2±6.6 (98.2) | 0.4 | 0.5 |
| DIA | 97±10 | 99±14 | 4.9±0.1 (98.3) | 25.0±1.6 (100.1) | 48.8±1.9 (97.5) | 94.6±7.8 (94.6) | 0.3 | 0.5 |
| DEA | 86±3 | 94±3 | 5.0±0.2 (99.5) | 25.3±1.3 (101.1) | 47.8±1.8 (95.7) | 97.3±9.3 (97.3) | 0.5 | 0.5 |
| ATZMM | **2 ng/mL** | **10 ng/mL** | **2 ng/mL** | **10 ng/mL** | **20 ng/mL** | **40 ng/mL** | | |
| | 96±5 | 93±5 | 2.0±0.0) (100.8.) | 10.0±0.3 (99.7) | 19.0±0.7 (95.0) | 39.0±2.1 (97.5) | 0.1 | 0.2 |
| ATZ | 102±4 | 93±5 | 2.0±0.0 (98.7) | 9.9±0.5 (98.6) | 19.2±0.8 (96.1) | 38.2±2.4 (95.6) | 0.1 | 0.2 |

(a) Recovery was calculated base on three replicate results of each analytes in each concentration

**Table 3: Method Precision**

| Analyte | QC Low | | QC Medium | | QC High | |
|---|---|---|---|---|---|---|
| | Mean (ng/mL) | RSD% | Mean (ng/mL) | RSD% | Mean (ng/mL) | RSD% |
| ATZ-OH | 4.9±0.7 | 14.1 | 10.4±1.3 | 12.4 | 57.7±6.5 | 11.2 |
| DACT | 5.5±1.1 | 20.5 | 12.2±1.4 | 11.8 | 64.8±9.1 | 11.1 |
| DEAMM | 5.3±0.8 | 14.7 | 11.5±0.9 | 7.9 | 57.7±4.8 | 8.2 |
| DIA | 5.3±0.6 | 12.1 | 10.5±0.6 | 5.3 | 51.2±4.2 | 8.3 |
| DEA | 4.7±0.5 | 10.2 | 10.0±0.6 | 5.6 | 50.6±4.8 | 9.4 |
| ATZMM | 5.1±0.5 | 9.9 | 10.5±0.4 | 4.1 | 51.8±4.5 | 8.6 |
| ATZ | 5.2±0.5 | 10.4 | 11.0±0.7 | 6.5 | 55.4±5.2 | 9.4 |

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