Assessment of Maternal Occupational Pesticide Exposures during Pregnancy and Three Children with Birth Defects: North Carolina, 2004

Occupational and Environmental Epidemiology Branch
Division of Public Health
North Carolina Department of Health and Human Services
Raleigh, North Carolina
May 18, 2006

Authors and Contact Information

Lead Authors: Ann N. Chelminski, MD, MPH and Sheila Higgins, RN, MPH

Contributing Authors: Robert Meyer, PhD, MPH, Douglas Campbell, MD, MPH, William J. Pate, MSPH, Ken Rudo, PhD

Author affiliations: Dr. Meyer is Director of the North Carolina Birth Defects Monitoring Program, State Center for Health Statistics, NC Division of Public Health. All other authors are in the Occupational and Environmental Epidemiology Branch, Epidemiology Section, NC Division of Public Health.

Contact: Ann Chelminski or Sheila Higgins
919-707-5900
Occupational and Environmental Epidemiology Branch
1912 Mail Service Center
Raleigh, NC 27699-1912

Introduction

In August of 2005, the North Carolina Division of Public Health, Occupational and Environmental Epidemiology Branch (OEEB) was notified that three women who had worked on farms in North Carolina owned by Ag-Mart had delivered infants with birth defects. All three births took place in Florida where the women also worked on Ag-Mart farms and lived near each other. This report summarizes the OEEB's investigation and assessment of the pesticide exposures likely experienced by these women while in North Carolina. The aim of this report is to summarize the authors' findings regarding the likely occupational pesticide exposures for each case-mother and the duration and timing during gestation of any exposure. Specifically, this report seeks to address the concern that pesticide exposures may have contributed to the birth defects seen in the children of the three case-mothers.

It is important to note that the authors of this report have relied upon information collected by the Florida Department of Agriculture and the Florida Department of Health/Collier County Health Department as OEEB did not have access to medical records for two of the three affected children. OEEB staff were able to interview two of the case-mothers; a description of these interviews will follow. Pesticide application records and work records were provided by Ag-Mart. The records note the date and field to which pesticides were applied and the approximate time of application. Work records note the date, time and approximate field locations of the women on days worked. The limitations of this report are addressed in the discussion and conclusions section.

Background

In December, 2004 and February, 2005 three babies were born in Immokalee, Florida (Collier County) with serious birth defects. Basic descriptive information for the mothers and their infants are presented in Table 1.

Table 1: Descriptive information for case mothers and infants

Case-mother#	Date of Delivery	Maternal Age (years)	Infant Sex and Birth Defect	Personal risk factors for birth defects
Case-mother 1	Dec. 17, 2004	19	Male born with no arms or legs (Tetra-melia).	None known
Case-mother 2	Feb. 4, 2005	30	Male with a diagnosis of Pierre Robin syndrome. Abnormalities include small jaw, high palate.	Father of baby has a small jaw (micrognathia). History of prior stillbirth.
Case-mother 3	Feb. 6, 2005	21	Female with multiple malformations: cleft lip and palate, lack of visible sex organs, solitary kidney. Died 3 days after birth.	One prior pregnancy with malformation, fetal death.

Source: "Investigation into the Occurrence of Congenital Malformation in Immokalee, Collier County, Florida 2005", Collier County Health Department report.

The mothers of all three case-infants are migrant farm workers from Mexico. Each mother worked before and during her pregnancy on farms owned by Ag-Mart, an agricultural operation based in Plant City, Florida. The mothers were employed to plant, tie and harvest grape tomatoes on farms in Florida and North Carolina.

An investigation was initiated by the Florida Department of Agriculture and Consumer Services (FDACS) on March 28, 2005, at two Ag-Mart farm locations. Pesticide application records and work records for the three case-mothers were collected. Violations of federal and state pesticide regulations were identified. The FDACS issued a Notice of Violations with proposed fines on October 12, 2005. The FDACS also prepared a report summarizing information available on the health effects of the pesticides used in fields where the cases worked in Florida. The final draft of "Teratogenic Potential of Pesticides Associated with Florida Ag-Mart Farm Worker Investigation" was completed on October 2, 2005 and was shared with the Florida Department of Health and other interested parties. A copy of that document (excluding pesticide application records) is attached to this report as Appendix A.

The Collier County (Florida) Health Department (CCHD) began an epidemiologic investigation of the birth defects cases in February 2005. CCHD staff had access to the medical records of the case-infants and interviewed the mothers and fathers of the affected children. Interviewers collected information on each parent's medical history, family history, nutritional habits, work habits, and possible exposure to alcohol, drugs, and medications. Their final report "Investigation into the Occurrence of Congenital Malformation in Immokalee, Collier County, Florida 2005" was shared with NC OEEB and others. A copy of that document is attached to this report as Appendix B.

On April 19, 2005, the North Carolina Department of Agriculture and Consumer Services Pesticide Section (NCDACS) received an investigation referral from the U.S. Environmental Protection Agency (EPA) Region IV office. The Pesticide Section is responsible for the administration and enforcement of the N.C. Pesticide Law of 1971 and pursuant regulations adopted by the N.C. Pesticide Board. This law is based on the stipulations outlined in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Pesticide Section activities include overseeing the registration of pesticides, licensing and certifying commercial and private pesticide applicators, assuring the proper handling, transportation, storage and disposal of pesticides, and inspections of sites where pesticides are used. The EPA referral asked NCDACS to investigate possible violations of pesticide regulations and pesticide exposures for the same three farm workers at the Ag-Mart facilities in Leland and Currie, North Carolina. On-site inspections at both sites by NCDACS began two days later.

On August 8, 2005 the OEEB in the North Carolina Division of Public Health was asked by NCDACS to evaluate possible pesticide exposures experienced by the women of concern and to assess any relationship between the possible exposures and the health effects seen in the affected children. OEEB staff within the Medical Evaluation and Risk Assessment Unit and the Occupational Health Surveillance Unit collaborated with

NCDACS and the North Carolina Birth Defects Monitoring Program to complete this assessment.

Methods and Data Sources

Medical Information for the children and their mothers:
The children of interest were all born in Florida between December, 2004 and February, 2005. OEEB obtained the medical information on two of the mothers and infants from the reports issued by FDACS ("Teratogenic Potential of Pesticides Associated with Florida Ag-Mart Farm worker Investigation" 10/02/2005) and the Collier County Health Department ("Investigation into the Occurrence of Congenital Malformations in Immokalee, Collier County, Florida 2005"). OEEB staff were able to review copies of the actual medical records for Case-mother 1 and to interview Case-mother 1 by telephone. It must be noted that this interview was conducted after she had engaged an attorney. OEEB staff also interviewed Case-mother 2 in person. Case-mother 3 is reported to be living in Mexico. Because of privacy concerns regarding the sharing of protected health information, OEEB staff have not had access to all of the medical records or the transcripts of interviews done in Florida for all three of the case-mothers.

Exposure Assessment:
Field assignments, work hours, and pesticide application records were provided to OEEB by NCDACS. OEEB also requested and obtained work records and field assignments for the case-mothers from Ag-Mart. Ag-Mart representatives state that the source of work dates and hours were the employee timecards. An initial note sent to OEEB by staff in the Human Resources office of Ag-Mart stated that the source of the workers' field assignments were crew leader assignments. Ag-Mart's president and attorneys state that the field locations provided to NCDACS and OEEB for each case-mother represent all possible fields where the women might have worked on a given date. They state that the fields listed as locations of work for the three workers were derived from records of harvested tomato arrival dates at the packing facility in Florida and from known tomato plantings and harvests. Neither the Worker Protection Standard nor labor regulations require that a grower maintain documentation of the specific fields in which agricultural workers work.

Spreadsheets were compiled for each of the three case-mothers summarizing work dates, times, and location for dates worked in North Carolina within the period of concern for each case pregnancy. The period of concern is defined in this report as the time period beginning three months prior to the estimated date of conception through the thirteenth week after conception. Only work days that fell within the period of concern were assessed.

For each case-mother, the date and hours for each day of work were noted. The work location was considered to be all of the fields listed on the record provided by Ag-Mart. The company is now disputing the accuracy of the field location information. Information on date, hours and location of work, as listed in records provided by Ag-Mart, was correlated with pesticide application records for each date. Because the work

records cannot exclude or confirm the field location of a farmworker on any specific workday, the authors of this report have assumed that a possible exposure occurred if the work time fell within the Restricted Entry Interval (REI) for a pesticide application made to any of the fields listed as a work location on the date of work. The REI is the period of time that fieldworkers are supposed to wait before re-entering a field after a pesticide application. The REI is designed to prevent exposures to pesticide residues at concentrations that pose a human health risk for field re-entry workers; it is calculated by the U.S. EPA during the registration process for individual pesticides. Appendix D lists the REIs for the pesticides included in this report.

Whether actual pesticide exposure occurred depends on multiple factors including the physical characteristics of the pesticide compounds, the effects of other chemical compounds present, pesticide residue levels in soil, air and on plants, the types of work performed, the clothing worn by the workers, the use of any protective equipment, actual field locations and weather conditions (e.g. heat, humidity) (DHHS, 2005). To acknowledge the uncertainty regarding exposure, hours worked within an REI will be referred to as "possible exposure."

Other than job descriptions noted on the Ag-Mart Company work schedule (e.g. "planting and tieing"), no information regarding use of personal protective equipment, hand washing, and other work practices was available to OEEB for Case-mother 3; information from Case-mothers 1 and 2 was obtained by interview.

Toxicological Data: Information for the pesticides of concern was obtained from the Florida Department of Agriculture and Consumer Services Report ("Teratogenic Potential of Pesticides Associated with Florida Ag-Mart Farmworker Investigation" – 10/2/05; Appendix A) and the TOMES® Plus System Database, 2005.

Epidemiological literature review: The Medline database was queried using the search terms "pesticides AND birth defects." The review was limited to English language articles and those that included birth defects as an outcome of pesticide exposure. Additional published papers were found from reference lists. As this was not a formal systematic review of the literature pertaining to maternal pesticide exposures and birth defects, no pre-defined selection criteria were used for the review of published papers.

NC Birth Defects Registry Data:
The NC Birth Defects Registry was searched by Robert Meyer, PhD of the North Carolina Birth Defects Monitoring Program (NCBDMP) for information on the prevalence of the types of birth defects seen in the case-infants in the counties where the case-mothers worked as compared to the statewide prevalence of these types of birth defects. The registry is a statewide, population-based surveillance system that collects information on all infants in North Carolina born with major birth defects. Registry data is collected by trained field staff who review and abstract data from all hospitals that provide labor and delivery and pediatric services, as well as from selected specialty clinics, and other facilities throughout the state. In order to be included in the registry, the infant must have been born to a resident of NC and be diagnosed with one or more

birth defects within the first year of life. The registry includes all live-born infants, fetal deaths, and pregnancy terminations regardless of gestational age. The NCBDMP uses the British Pediatric Association (BPA) coding system. Data on maternal exposures to known or suspected teratogens are also collected from the medical record when available, however, such information is generally of limited use due to the inconsistent and incomplete documentation of such exposures in patient records.

Data Analysis: As there is no comparison group, this report is a descriptive analysis.

Results

Regulatory Investigation

From April 21 to April 25, 2005 the NCDACS Pesticide Section inspected pesticide use at the Ag-Mart farms in Pender and Brunswick counties. The Pesticide Section also obtained pesticide application records and work records for the case-mothers for the time period in 2004 when the women worked in North Carolina. Based on their inspections of Ag-Mart's farm sites, record review, interviews with farm management, and interviews with several Ag-Mart employees, the Pesticide Section investigators found evidence of numerous violations of regulations relating to pesticide use, including the Worker Protection Standard (WPS). Cited violations consisted of:

- Label violations
- WPS violations in the areas of: provision of information about applications, field entry restrictions, pesticide safety training for workers and handlers, notice of application, knowledge of handling and site-specific information, safe operation of equipment, and decontamination.
- Disposal violations
- Storage requirement violations

Label violations consisted mostly of REI violations. They also included lack of compliance with pre-harvest intervals, prohibited mixtures of certain pesticides, and incidents of over-application in 2004 (e.g. Monitor was applied 14 times at one NC site and 16 times at the other NC site; only 5 applications per season are permitted). WPS violations were significant. After application there was no required display of information about applied pesticides and workers were allowed to work in the fields prior to REI expiration. No personal protective equipment (gloves, coveralls, etc.) was provided to workers re-entering fields within the REI. There was evidence that training was given by unqualified instructors, that the wrong type of training was provided to handlers, and that Ag-Mart management was not aware of training requirements. There was a lack of double (oral and written) notification of pesticide applications. Workers interviewed stated that although they were told to apply pesticides, they did not have access to pesticide labels or have knowledge about application or proper use of required equipment. There was no decontamination material available nor was there enough water for worker decontamination or drinking water. Disposal violations consisted of incidents of open burning of pesticide containers. Ag-Mart violated storage requirements by storing a container of gasoline in the pesticide storage area. For the time period that the

case-mothers worked in North Carolina in 2004, both Ag-Mart sites' records, "show that these workers re-entered the fields before the expiration of the REI on multiple occasions" (Appendix C).

NCDACS delivered a Notice of Violation to Ag-Mart's Regional Manager in North Carolina on October 21, 2005. The Notice includes 369 alleged pesticide violations with fines totaling $184,500. The complete Notice of Violation is included in this report as Appendix C.

The most recent inspection of Ag-Mart performed by North Carolina, Department of Labor Occupational Safety and Health Administration (NC DOL OSHA) was in 2003. This inspection was prompted by a complaint about field sanitation. There were multiple citations issued under the Hazard Communication Standard (1910.1200) and the Temporary Labor Camps Standard (1910.142). Violations of the Hazard Communication Standard included: migrant farm worker employees mixing and applying pesticides (e.g. Kocide, Dithane M-45 (mancozeb), Agrimek, and Ecozin) were not supplied with adequate personal protective equipment as required by the Material Safety Data Sheets (MSDS) for the chemicals being mixed and applied, lack of a written hazard communication program, lack of MSDS information in the workplace, lack of employee training at required times, and lack of training on label information. Violations regarding temporary labor camps were numerous and included lack of proper sewage processing and fire safety compliance. There was no compliance with preoccupancy inspection applications. In 2005 NC DOL OSHA conducted a housing inspection. It was determined that worker housing was unregistered and that workers were staying in a motel. NC DOL OSHA is still working on this investigation; a report is pending.

Assessment of Pesticide Exposure in Case Homes

In an effort to evaluate possible pesticide exposures at the three women's homes, the North Carolina Structural Pest Control Division sent a field investigator to interview the regional manager for Ag-Mart in order to get physical addresses for the case-mothers. The manager reported that Ag-Mart did not provide housing for their workers in North Carolina. Payroll records were searched in an attempt to get addresses for the case-mothers in North Carolina, but only the addresses of the crew leaders were found. Further attempts by the field investigator to confirm the housing locations of the case-mothers were unsuccessful.

Mutagenic/Teratogenic Potential of Pesticides used in North Carolina

The TOMES® database, the FDACS report (Appendix A), and studies published in the medical literature were reviewed for evidence regarding the mutagenic and teratogenic potential of each of the pesticides to which the case-mothers were potentially exposed in North Carolina. Based on this review, the following pesticides were found to have evidence of teratogenicity: Agri-Mek 0.15 EC Miticide/Insecticide (abamectin and n-methylpyrrolidine), Dithane M45 (mancozeb), Kocide 101 (copper hydroxide), Monitor 4 Spray (methamidophos), and Penncozeb 80 WP. Dithane M45 and Penncozeb are ethylene bisthiocarbamate pesticides; a metabolite and degradation product of these pesticides is ethylene thiourea. Of these compounds, Dithane M45, Monitor 4 Spray and

Penncozeb are also considered to be mutagenic. The pesticide Danitol 2.4 EC was evaluated even though the active ingredient, fenpropathrin, is not considered to be a teratogen. Two inactive ingredients in Danitol 2.4 EC, naphthalene and 2-ethylhexanol, are considered to be teratogenic in animal studies. A detailed discussion of the teratogenicity of these pesticides, their active and inactive teratogenic ingredients, and their teratogenic metabolic or degradation products are detailed in the FDACS report attached to this report as Appendix A. A list of the pesticides included in this report and their respective Restricted Entry Intervals (REIs) is attached as Appendix D.

Time Worked in North Carolina and Period of Concern for Teratogenic Exposures

Table 2 presents the dates worked in North Carolina in 2004 for each of the case-mothers. Also shown is the period of concern. In this report, the period of concern is defined as the period of time three months before the earliest date in the range given for probable date of conception (DOC) through the thirteenth week after the latest date in the DOC range. The range of dates for the probable DOC was provided by the CCHD report (Appendix B). This period of concern is similar to the Critical Gestational Period used by authors of the CCHD report in that it reflects the period of greatest vulnerability of the fetus to a teratogenic exposure. The dates used in this report differ from those used in the CCHD report because we chose a broader window of time for the period of concern because of the uncertainty in the dating of the pregnancies and in order to include the pre-conception period. OEEB was not able to obtain pesticide exposure information for the case-fathers.

Table 2: Time Worked in NC within Gestational Period of Concern

	Case-mother 1	Case-mother 2	Case-mother 3
Probable DOC* (range)	4/03/04 - 4/17/04	4/10/04 - 4/24/04	5/16/04 - 5/30/04
Period of concern†	1/3/04 - 7/10/04	1/10/04 - 7/16/04	2/16/04 - 9/05/04
Dates worked In NC in 2004	4/19/04 - 10/02/04	6/14/04 - 11/12/04	9/13/04 - 10/22/04
Dates worked In FL, 2004	2/1/04 - 4/19/04	2/1/04 - 5/31/04	3/7/04 - 4/03/04 and 6/05/04 - 6/21/04
Dates in NC Within period of concern	4/19/04 - 7/10/04	6/14/04 - 7/16/04	None
Estimated gestational ages (days after DOC)	9-91	59-89	N/A

*DOC=Date of conception. See CCHD report, p.5

[†]Defined as 3 months prior to earliest date in range of DOC to end of 13th week after latest date in DOC range.

As Table 2 shows, Case-mother 1 spent the most time (almost three months) in NC during her period of concern. Case-mother 2 worked approximately one month in NC during her period of concern. Case-mother 3 worked in NC later in her pregnancy, after the end of the period of concern. Because Case-mother 3 did not work in NC during the period of concern for her pregnancy, this report will focus on possible exposures to pesticides experienced by Case-mother 1 and Case-mother 2 in North Carolina. All three case-mothers did work in Florida during part of their period of concern (as defined above). It is the goal of this report to assess pesticide exposures that occurred in North Carolina during the period(s) of concern.

Routes of exposure

The most significant route of exposure to pesticides for fieldworkers doing hand labor is dermal absorption of pesticide. The concentration of pesticide that can be dermally absorbed depends in part on the amount of pesticide residue on the foliage that can be dislodged and on the fieldworker's use of gloves or other personal protective equipment (PPE) (Fenske, 1997). PPE is required for workers re-entering a field prior to the end of the REI. No use of PPE is reported for the three workers who are the focus of this report. The oral and inhalation routes of exposure may also be significant. Under favorable weather conditions, pesticide residues may become airborne and could be inhaled. Exposure could occur via ingestion if pesticide-contaminated food or drink was consumed. One way this could occur is if the women did not wash their hands before eating, either at work or at home. In the NC DACS Notice of Violation, a lack of hand washing facilities at one of the farm sites in NC was noted. Additional citations include inadequate labeling and disposal of pesticide containers and failure to provide the amount of drinking water required for each farm worker (Appendix C).

Possible Exposures for Case-mother 1 in North Carolina

Case-mother 1 worked in North Carolina from 4/19/04 - 10/02/04. The period of concern for Case-mother 1 is 1/03/04 - 7/10/04, with an estimated date of conception of approximately 4/10/04. The portion of time this mother worked in NC within the period of concern is two months, 21 days. Before coming to North Carolina, Case-mother 1 worked in Florida, from 2/1/04 - 4/19/04. Possible pesticide exposures in Florida are discussed on pages 55-56 of the FDACS report (Appendix A). Three pesticides used by Ag-Mart in Florida were not applied to fields within three days of a work date during the period of concern for Case-mother 1 in NC; these are Asana XL, Courier, and Thionex 3 EC. Table 3 shows the pesticides applied to fields in which Case-mother 1 was assigned to work at two Ag-Mart farms in eastern North Carolina. As shown in Table 3, there were multiple dates on which Case-mother 1 was assigned to work in a field at a time within the REI for a recently applied pesticide. Those hours worked prior to the expiration of the REI for a pesticide are noted separately and are also included in the total hours of work. Whether the REI for Oxidate was violated is uncertain since the REI ends when the product is "dry." As the pesticides were often applied as mixtures, exposure to multiple pesticides within the REI for one or more pesticides is possible. Early field re-entry possibly took place after the application of a number of pesticides with mutagenic

and/or teratogenic effects in animal testing; these include Danitol, Dithane M45, Kocide 101, Monitor, and Penncozeb. On February 6, 2005, this mother gave birth to a male child born with no arms or legs (tetramelia).

Table 3: Summary of Possible Exposures for Case-mother 1 by Pesticide during Period of Concern

Pesticide	Teratogenic/ mutagenic effects in animal studies	Ingredient(s)	Work Hours in Possible REI* Violation	Total hours worked†
AzaDirect	No	Azadirachtin	16	87
Bravo	No	Chlorothalonil	34	34
Champion	No	Copper hydroxide	22	56
Danitol	Yes	Fenpropathrin Naphthalene 1,2,4-Trimethylbenzene 2-Ethylhexanol	16	16
Dipel DF	No	*Bacillus thuringiensis*	12	68.5
Dithane M45	Yes	Ethylene bisdithiocarbamate Manganese, Zinc	18	58.5
Entrust	No	Spinosad	4	46
Kocide 101	Yes	Copper hydroxide	40.5	73
Monitor	Yes	Methamidophos	16	16
Oxidate	No	Hydrogen peroxide	?	8
Penncozeb 80WP	Yes	Mancozeb (Ethylene bisdithiocarbamate, Mg, Zn, Ethylene thiourea (trace)	31.5	41.5
Serenade	No	*Bacillus subtilis*	12	79
Spintor	No	Spinosad	12	31
Xentari	No	*Bacillus thuringiensis*	22.5	48.5

*REI = Restricted Entry Interval – that time period

†includes all hours worked in a field to which the pesticide of interest had been applied within 3 days prior to work. Includes hours worked in violation of an REI.

Case-mother 1: Known Risk Factors for Birth Defects

No known personal risk factors for having a child with a birth defect are reported for Case-mother 1. She was age 19 at the time of her child's birth and was pregnant for the first time. A physician in OEEB reviewed the medical records for her child. Her child was born with all limbs missing (a small section of bone is present in the left upper extremity). Based on an ultrasound exam at approximately the 28th week of gestation, the gestational age at delivery was determined to be 36 5/7 weeks. No significant maternal medical history, family history or use of alcohol, tobacco or drugs is noted in the medical records. Pertinent laboratory results include a normal newborn screen report, a normal

male chromosome analysis (46, XY), and normal routine prenatal lab results including syphilis and hepatitis serologies. Case-mother 1 reported to Florida investigators that she did not consume alcohol or illicit drugs, take herbal/folk remedies or medications, or use tobacco during her pregnancy. She apparently took prenatal vitamins beginning in the fourth month of pregnancy and had a balanced diet (Table 4, p.7, Appendix 2).

Case-mother 1: Telephone Interview

On 3/7/06 OEEB staff interviewed Case-mother 1 by telephone in Spanish. At the time of this interview she had retained legal counsel and her attorney was present, but did not answer questions for her. In the interview, she denied the use of prescription, over-the-counter, or traditional/folk/herbal medicines during her pregnancy. She denied using tobacco, alcohol or illicit drugs during her pregnancy. She stated that she began taking prenatal vitamins during month four of her pregnancy when she began prenatal care. She denied a family history of birth defects or a personal history of prior pregnancy. She denied any blood relationship (consanguinity) with the child's father. She stated that she and the child's father were both fieldworkers for Ag-Mart and that she had experienced direct spray as well as drift from pesticide applications while working for Ag-Mart. She denied receiving verbal or written (posted) warnings not to enter a field because of a recent pesticide application. She stated that she was not provided with gloves, coveralls or other personal protective equipment while working for Ag-Mart in North Carolina.

Case-mother 1: Possible pesticide exposures in Florida

Case-mother 1 worked on Ag-Mart farms in Florida from 2/01/04 - 4/17/04. Most of this period was pre-conception, as conception is estimated to have occurred between 4/03/04 - 4/17/04 (CCHD report, p. 5). The Collier County Health Department (CCHD) report does not include potential exposures for Case-mother 1 as she was not in Florida during the time period that the authors of that report define as the critical gestational period, 4/21/04 -6/02/04. In records provided with the FDACS report, there appear to be several dates in the pre-conception period during which Case-mother 1 worked in violation of the REI for Monitor 4 spray, an organophosphate.

Case-mother 1: Estimated gestational age on dates of work

Table 4 includes the estimated gestational age in days for each date on which Case-mother 1 worked. As noted previously, the gestational age was estimated by using the date in the middle of the range of dates provided by the CCHD report for dates of conception. For Case-mother 1, the gestational age is calculated as the number of days after April 10, 2004 and is noted in bold print. Hours of work in possible violation of the REI for a particular pesticide are in bold as well. Hours of work in plain type include any hours in possible violation of the REI as well as other hours worked within three days of a pesticide application. Whether mutagenic and/or teratogenic effects have been reported in the offspring of exposed animals in animal testing of individual pesticides is noted.

Table 4: Estimated Gestational Age on Dates of Work (Exposure) for Case-mother 1

Pesticide	Teratogenic/ mutagenic effects in animal studies	**Estimated gestational age in days on work date** (#hours work/ **# hours in possible violation of REI**)	Total hours in possible violation of REI*	*Total hours worked*
AzaDirect	No	**32** (6), **34** (6/4), **51** (8), **53** (8), **54** (7/4), **56** (10), **68** (7), **79** (9/4), **82** (10), **83** (10/4), **84** (6)	16	*87*
Bravo Weather Stik	No	**53** (8/**8**), **83** (10/**10**), **84** (6/**6**), **88**(10/**10**)	34	*34*
Champion	No	**32** (6), **34** (6/**6**), **51** (8), **53** (8), **54** (7/**7**), **75** (12), **79** (9/**9**)	22	*56*
Danitol	Yes	**84** (6/**6**), **88** (10/**10**)	16	*16*
Dipel DF	No	**12** (9/**4**), **14** (8.5), **15** (6), **25**(9/**4**), **56** (10), **68** (7), **79** (9/**4**), **82** (10)	12	*68.5*
Dithane M45	Yes	**12** (9/**9**), **14** (8.5), **15** (6), **51** (8), **53** (8), **79** (9/**9**), **82** (10)	18	*58.5*
Entrust	No	**53** (8), **68** (7), **75** (12), **79** (9/**4**), **82** (10)	4	*46*
Kocide 101	Yes	**12** (9/**9**), **14** (8.5), **15** (6), **25** (9/**9**), **30** (2.5/**2.5**), **32** (6/**6**), **34** (6/**6**), **51** (8/**8**), **53** (8), **56** (10)	40.5	*73*
Monitor	Yes	**84** (6/**6**), **88** (10/**10**)	16	*16*
Oxydate	No	**51** (8/? REI ends when product is dry)	?	*8*
Penncozeb 80 WP	Yes	**25** (9/**9**), **30** (2.5/**2.5**), **32** (6/**6**), **34** (6/**6**), **51** (8/**8**), **56** (10)	31.5	*41.5*
Serenade	No	**32** (6), **34** (6/**4**), **51** (8), **53** (8), **54** (7/**4**), **68** (7), **75** (12), **79** (9/**4**), **82** (10), **84** (6)	12	*79*
Spintor 2SC	No	**25** (9/**4**), **32** (6/**4**), **51** (8), **53** (8/**4**)	12	*31*
Xentari	No	**30** (2.5/**2.5**), **32** (6), **34** (6/**4**), **51** (8/**4**), **83** (10/**4**), **84** (6/**4**), **88** (10/**4**)	22.5	*48.5*

*REI: Restricted Entry Interval

Case-mother 1: Assessment of the Relationship between Possible Exposures and Birth Defects

Case-mother 1's child was born without upper or lower limbs (tetramelia). Conditions associated with congenital limb deficiencies include chromosomal abnormalities (6%), single gene mutations and inherited syndromes (24%), vascular insufficiency (35%), maternal diabetes, prenatal alcohol use and the prenatal use of teratogenic medications including warfarin (an anticoagulant), phenytoin (an anticonvulsant) and thalidomide (Stevenson, 1993; Holmes, 2002). Thalidomide was prescribed, mainly in Europe, as a

sedative and for relief of nausea in the 1950's. It caused a variety of limb defects in infants whose mothers took the drug at a specific time during gestation. Complete absence of a limb (amelia) was not the defect most commonly associated with thalidomide use. The majority of those with thalidomide-induced abnormalities of the arms had normal legs (Holmes, 2002). In the U.S., thalidomide's current use in the treatment of leprosy and other specific conditions is closely monitored by the Food and Drug Administration and the licensed manufacturer (www.fda.gov).

It is estimated that 32% of congenital limb deficiencies are due to unknown causes (Makhoul, 2003). Upper and lower limb formation occurs between weeks 4-8 of gestation or gestational days 28-56. The uncertainty regarding the dating of these pregnancies must be considered when assessing the timing of possible exposures. During estimated gestational days 28-56, Case-mother 1 had possible exposures to Dipel DF, Dithane M45, Entrust, Kocide 101, Oxidate, Penncozeb 80 WP, Serenade, Spintor 2SC and Xentari. Of these, Dithane M45, Kocide 101 and Penncozeb have shown developmental effects in animal testing. Kocide 101 contains the active ingredient copper hydroxide. The toxicology of Kocide 101 is described on pages 49-51 of the FDACS report. While delayed growth and reduced bone ossification were noted in the offspring of rats exposed to dietary copper, other rat studies showed no difference in copper-exposed rats compared to controls. Mice and hamsters injected with copper as copper sulfate or copper citrate bore offspring with a variety of defects including tail defects (FDACS report, p. 51).

Dithane and Penncozeb both contain the fungicide mancozeb. A product of the degradation and metabolism of mancozeb is ethylene thiourea (ETU). According to the Material Safety Data Sheet (MSDS) for Penncozeb, ETU is on California's Prop 65 List of Developmental Toxins and on the "Right to Know" lists for Massachusetts, New Jersey, and Pennsylvania. The teratogenicity studies of ETU in multiple species of animals are detailed in pp. 36-44 of the FDACS report. Limb defects have been seen in the offspring of animals dosed with both ETU and the parent compound mancozeb. Skeletal malformations, including missing bones, have been observed in the offspring of rats dermally exposed to ETU without observable toxicity in the dams (FDACS report,p. 37). A June 2005 risk assessment by the U.S. Environmental Protection Agency (EPA) on mancozeb and ETU looked at developmental effects of exposure to both compounds. Dermal absorption for ETU is 26% while that of mancozeb is only 1%. Inhalational absorption is 100% for both compounds. The adverse effects from these two exposure routes are considered by EPA to be similar for similar durations of exposure. The most sensitive endpoints selected by EPA for their risk assessment consisted of the thyroid as the endpoint for mancozeb and developmental and thyroid effects as the endpoints for ETU. According to this risk assessment, there is a lack of data on the acute developmental neurotoxicity of both mancozeb and ETU. Adverse developmental effects seen with both compounds include hydrocephaly and related lesions, skeletal system defects, and other defects. Birth defects were seen in offspring of exposed animals at doses which only caused weight gain and decreased food consumption in the mothers (EPA, 2005).

Between gestational days 28-56 (period of limb formation), Case-mother 1 had possible exposures to Dithane (mancozeb), Kocide and Penncozeb as well as to other pesticides not known to be mutagenic or teratogenic. None of the possible exposure to Dithane M45 during this time period was in violation of the REI; possible exposure to Dithane M45 did occur on gestational days 12 and 79 when Case-mother 1 worked nine hours (on each of these days) within possible violation of the REI for Dithane. A total of 22.5 hours were worked in possible violation of the REI for Penncozeb during this time period. Possible exposures to multiple pesticides occurred on gestational days 30, 32, 34, 51 and 53 as detailed in Table 4. Exposure to pesticide mixtures was also possible on gestational day 25, when Case-mother 1 worked in apparent violation of the REI for Dipel DF (4 hours worked), Kocide 101 (9 hours), Penncozeb (9 hours) and Spintor 2SC (4 hours).

Possible Exposures for Case-mother 2 in North Carolina

Case-mother 2 worked in North Carolina from June 14 - November 12, 2004. The portion of this time that falls within the period of concern for her pregnancy is approximately one month, June 14 - July 16, 2004. Before working in North Carolina, Case-mother 2 had worked for nearly four months on Ag-Mart farms in Florida. On February 4, 2005, in Florida, she gave birth to a child with an underdeveloped jaw (micrognathia), a high arched palate and several additional minor abnormalities. The child was diagnosed with Pierre Robin Syndrome.

Table 5 summarizes the agricultural pesticides to which Case-mother 2 was possibly exposed in NC during the period of concern for her pregnancy. Hours of work within an apparent REI violation are noted as well as total hours of work on the date of interest.

(See Table 5 on following page)

Table 5: Summary of Possible Exposures for Case-mother 2 by Pesticide during Period of Concern

Pesticide	Teratogenic/ mutagenic effects in animal studies	Ingredient(s)	Work Hours in possible REI* Violation	Total hours worked†
Agrimek 0.15EC	Yes	Avermectrin-B1 Butylated hydroxytoluene n-Methylpyrrolidone	8	33
AzaDirect	No	Azadirachtin	4	85
Bravo	No	Chlorothalonil	8	51
Champion	No	Copper hydroxide	21	94
Danitol	Yes	Fenpropathrin Naphthalene 1,2,4-Trimethylbenzene 2-Ethylhexanol	8	33
Dipel DF	No	*Bacillus thuringiensis*	8	104
Dithane M45	Yes	Ethylene bisdithiocarbamate Manganese, Zinc	10	36
Entrust	No	Spinosad	4	62
Kocide 101	Yes	Copper hydroxide	0	20
Monitor	Yes	Methamidophos	16	33
Penncozeb 80WP	Yes	Mancozeb (Ethylene bisdithiocarbamate, Mg,Zn) Ethylene thiourea (trace)	8	53
Serenade	No	*Bacillus subtilis*	4	116
Xentari	No	*Bacillus thuringiensis*	4	48

*Restricted entry interval

†Includes hours in REI violation as well as other work hours within 3 days of pesticide application to the field.

Case-mother 2 spent a possible total of 103 hours working in possible violation of the REIs for the pesticides listed in Table 5. A possible 50 hours of this total were hours within the REI for pesticides that have shown mutagenic and/or teratogenic effects in animal testing--Agrimek, Danitol, Dithane M45, Kocide 101, Monitor and Penncozeb.

Case-mother 2: Known Risk Factors for Birth Defects

The father of Case-infant 2 has "significant micrognathia" according to the CCHD report (p. 4, Appendix 2). Also, this mother apparently had a previous pregnancy which ended in a stillbirth. Case-mother 2 was age 30 at the time of her affected child's birth, and

denied the use of folk or herbal medications, tobacco, caffeine or alcohol to Florida investigators. She reportedly took prenatal vitamins and had a balanced diet. She experienced nausea and "morning sickness" during pregnancy (Table 4, p.7, Appendix 2). OEEB authors were not able to review the medical records for this mother or her child. The CCHD report authors note that maternal blood tests were negative for evidence of acute infection with cytomegalovirus, rubella, toxoplasmosis or herpes simplex virus.

Case-mother 2: Interview

On May 17, 2006, OEEB staff interviewed Case-mother 2 with the assistance of two Spanish translators. This mother confirmed that she worked for Ag-Mart in Florida and North Carolina in 2004. Prior to working for Ag-Mart, she worked in agriculture in Mexico. Her husband also worked for Ag-Mart as a fieldworker in 2004. She stated that neither of them ever mixed or applied pesticides. She reported that while working, she typically wore long pants, a long-sleeved shirt and latex gloves that she bought for herself. She denied ever being provided with personal protective equipment at work. When asked about written or verbal notification of pesticide applications, she stated that she never saw signs and was never told when a field had been sprayed, but was sometimes told by her crewleader when she could re-enter a field. She has had five pregnancies and has four living children. She stated that none of the other children have birth defects. One pregnancy ended in stillbirth; that child did not have any obvious birth defects. She denied taking any prescription, herbal, over-the-counter, or traditional medications during pregnancy except for prenatal vitamins which she began when five months pregnant (after her initial prenatal care visit). She denied the use of tobacco, alcohol or illicit drugs during her pregnancy. When asked about illnesses during pregnancy, she denied any illness except fatigue and nausea. Her husband was not present during the interview, but she confirmed that he has a small chin and this is a feature shared by others in his family. She denied a family history of birth defects and denied any blood relationship (consanguinity) with her husband.

Case-mother 2: Possible pesticide exposures in Florida

According to the CCHD report, Case-mother 2 had an estimated DOC between 4/10/04 - 4/24/04 and a delivery date of 2/4/2005; these dates give a gestational length of 43-45 weeks (post-term). This mother worked in Florida in 2004 from February 1st to May 31st. During the two months post-conception she had eight cumulative days of possible exposure to pesticides in Florida including Echo 720 (chlorothalonil), Danitol 2.4 EC spray, Monitor 4 Spray, Kocide 101 and Lannate (methomyl). One of these days included possible exposure during REIs. Possible exposure to Kocide and Lannate occurred on one day (gestational day 45) within this two-month period. Pre-conception exposures were not included in this report, but records sent to OEEB by FDACS show that Case-mother 2 possibly entered fields in violation of the REI for Monitor 4 Spray during the three months prior to conception. See pages 56-57 of the FDACS report (Appendix 1).

Case-mother 2: Estimated gestational age on dates of work

Table 6 includes the estimated gestational age in days for each date on which Case-

mother 2 worked in a field. As noted previously, the gestational age was estimated by using the date in the middle of the range of dates provided by the CCHD report for dates of conception. For Case-mother 2, the gestational age is calculated as the number of days after April 17, 2004 and is noted in bold print. Hours of work in possible violation of the REI for a particular pesticide are also in bold print. Hours of work in plain type include any hours in possible violation of the REI as well as other hours worked within three days of a pesticide application. Whether mutagenic and/or teratogenic effects have been reported in the offspring of exposed female animals in animal testing is noted for each pesticide.

Table 6: Estimated Gestational Age on Dates of Work (Exposure) for Case-mother 2

Pesticide	Teratogenic /mutagenic effects in animal studies	**Estimated gestational age in days on date of work** (#hours work/ **# hours in possible violation of REI**)	Total hours in possible violation of REI*	*Total hours worked*
Agrimek	Yes	**86 (**8/**8), 87** (8), **88** (9), **89** (8)	8	*33*
AzaDirect	No	**59** (9), **60** (10), **61** (7), **72** (10/**4**), **73** (10), **74** (7), **75** (9), **77** (7), **79** (8), **87** (8)	4	*85*
Bravo Weather Stik	No	**73** (10), **79** (8), **86** (8/**8**), **87**(8), **88** (9), **89** (8)	8	*51*
Champion	No	**59** (9), **60** (10), **61** (7), **65** (11/**11**), **66** (10), **67** (9), **68** (12), **72** (10/**10**), **79** (8), **87** (8)	21	*94*
Danitol	Yes	**86** (8/**8**), **87** (8), **88** (9), **89** (8)	8	*33*
Dipel DF	No	**59** (9), **60** (10), **61** (7), **65** (11/**4**), **66** (10), **67** (9), **68** (12), **72** (10/**4**), **73** (10), **74** (7), **75** (9)	8	*104*
Dithane M45	Yes	**72** (10/**10**), **73** (10), **74** (7), **75** (9)	10	*36*
Entrust	No	**59** (9), **60** (10), **61** (7), **72** (10/**4**), **73** (10), **74** (7), **75** (9)	4	*62*
Kocide 101	Yes	**72** (10), **73** (10)	0	*20*
Monitor	Yes	**86** (8/**8**), **87** (8/**8**), **88** (9), **89**(8)	16	*33*
Penncozeb 80 WP	Yes	**72** (10), **73** (10), **86** (8/**8**), **87** (8), **88** (9), **89** (8)	8	*53*
Serenade	No	**59** (9), **60** (10), **61** (7), **66** (10), **67** (9), **68** (12), **72** (10/**4**), **73** (10), **74** (7), **75** (9), **77** (7), **79** (8), **87** (8)	4	*116*
Xentari	No	**77** (7), **79** (8), **86** (8/**4**), **87** (8), **88** (9), **89** (8)	4	*48*

*REI = restricted entry interval

Case-mother 2: Assessment of the Relationship Between Possible Exposure and Birth Defects

Pierre Robin syndrome is believed to occur due to inadequate jaw development that occurs before the ninth week of gestation. During normal development, the tongue lies between the developing palate "shelves" until it is drawn downwards during the tenth and eleventh weeks of gestation, allowing normal closure of the palate. In Pierre Robin syndrome, the small jaw displaces the tongue such that the tongue is not drawn down, inhibiting palatal closure (Stal, 2004). While case-infant 2 did not have cleft palate, he did have a high, arched palate. Pierre Robin syndrome is one of a number of craniofacial syndromes; some syndromes such as Velocardiofacial Syndrome have been linked to specific gene mutations (Stal, 2004).

As noted in Table 6 above, Case-mother 2 was at approximately day 59 of gestation when she began work in North Carolina in 2004. The time around gestational days 56-62 (week 9 of gestation) is the critical period when a teratogenic exposure is most likely to influence jaw development in the fetus. Case-mother 2 had possible exposures to Aza-Direct, Champion, Dipel DF, Entrust and Serenade during this time period while working in North Carolina. If two weeks before and after gestational days 56-62 are examined to allow for the uncertainty in the gestational age, then Case-mother 2 had possible exposure to almost all of the pesticides listed in Table 6 around the time of jaw formation. As previously noted, prior to working in North Carolina she worked in Florida and had possible exposures to the following pesticides: Agrimek 0.15EC, Asana XL, Aza-Direct, Danitol 2.4EC, Dipel DF, Echo 720, Kocide 101, Monitor 4 Spray, Omni supreme spray, Penncozeb 80 WP, and Thionex 3EC (CCHD Report, Table 3). Ethylene thiourea (ETU), a metabolic degradation product and trace component of Penncozeb, has caused micrognathia and cleft palate in the offspring of rats exposed orally to 80 mg/kg/day. In one study by Khera et. al this dose was not associated with any maternal effect, but in another study by Chernoff et al, a decrease in maternal weight and increased death was seen with this dose (Khera, 1973; Chernoff, 1979; EPA documents).

Birth Defects Prevalence in Brunswick and Pender Counties, North Carolina

Ag-Mart farms in North Carolina are located in Pender and Brunswick counties, adjacent counties in southeastern NC. The women of interest to this investigation worked at both farm sites. The North Carolina Birth Defects Monitoring Program database was searched in order to assess whether children residing in these counties are at higher risk for certain congenital malformations than children in other parts of the state,. A comparison between parents employed in agriculture and parents with non-agricultural occupations could not be made because this information is not consistently found in the database. Table 7 shows the five-year prevalence of congenital malformations by organ system for Brunswick and Pender counties and for the state overall for infants born in 1999-2003 (the most recent years that complete surveillance data are available). During that period there were 4,263 live births in Brunswick County and 2,366 in Pender County. Together these counties accounted for just over one percent of the 587,713 resident live births in North Carolina in 1999-2003. As shown in Table 7, the confidence intervals for each of the birth defect categories in Brunswick and Pender counties overlap the point estimates

for the state, suggesting that there is no significant difference between these counties and the state in the prevalence of birth defects by organ system.

Table 7: Prevalence of Major Birth Defects by Organ System, Brunswick County, Pender County and North Carolina, 1999-2003

Brunswick County (n=4,263 live births)

System	No. Cases	Prevalence*	95% CI**
CNS	15	35.2	19.7, 58.0
Cardiovascular	73	171.2	134.5, 214.8
Respiratory	14	32.8	18.0, 55.0
Orofacial	10	23.5	11.3, 43.1
Gastrointestinal	29	68.0	45.6, 97.6
Genitourinary	41	96.2	69.1, 130.3
Musculoskeletal	46	107.9	79.1, 143.7
Chromosomal	10	23.5	11.3, 43.1
All Birth Defects	202	473.8	412.0, 542.0

Pender County (n=2,366 live births)

System	No. Cases	Prevalence	95% CI
CNS	6	25.4	9.3, 55.1
Cardiovascular	42	177.5	128.2, 239.2
Respiratory	9	38.0	17.4, 72.1
Orofacial	5	21.1	6.9, 49.2
Gastrointestinal	13	54.9	29.3, 93.8
Genitourinary	30	126.8	85.7, 180.5
Musculoskeletal	32	135.2	92.7, 190.4
Chromosomal	7	29.6	11.9, 60.9
All Birth Defects	116	490.3	406.8, 585.1

North Carolina (n=587,713 live births)

System	No. Cases	Prevalence
CNS	1,904	32.4
Cardiovascular	8,417	143.2
Respiratory	1,907	32.4
Orofacial	897	15.3
Gastrointestinal	2,947	50.1
Genitourinary	7,514	128.3
Musculoskeletal	5,967	101.5
Chromosomal	1,314	22.8
All Birth Defects	26,641	453.3

*number of cases per 10,000 live births

**95% confidence interval, based on exact binomial limits

Because Pierre Robin sequence and limb reduction defects are two phenotypes of particular interest with regard to this investigation, the NCBDMP clinical database was queried for all such cases that have accrued from 2003 through December 2005 (note that data for 2004 and 2005 are still incomplete as of this writing). Statewide, there were 21 reported cases of PRS in the database, none of which were residents of Brunswick or Pender County. There were 99 infants with reported limb deficiency defects (BPA codes 755.200-755.499) statewide and three of the cases were from Brunswick or Pender County. One of these cases had a limb deficiency due to amputation caused by an amniotic band; the other two had reduction defects (missing fingers or toes) that most likely arose from different mechanism(s). From the information available, there is nothing to link the three cases together with regard to possible exposures or other clear risk factors.

Limitations of the data include the fact that only residents of North Carolina are captured in the NCBDMP database. As a result, cases of children with birth defects born to parents who are migrant farmworkers may not be captured by the database unless the parents list NC as their state of residence. Also, the database seldom captures information on parental occupation. It is not possible to compare rates of birth defects in children of agricultural workers to rates in children of parents not employed in agriculture because information on occupation is not collected for the majority of cases. While prevalence rates can be compared by ethnicity of cases (Hispanic/Latino compared to non-Hispanic/Latino), this comparison would still not include children with birth defects born to seasonal farmworkers whose residence is in another state. The data available cannot provide an assessment of rates of birth defects in women employed in agriculture as compared to those not employed in agriculture in North Carolina.

Epidemiologic studies of pesticide exposure and birth defects

Numerous epidemiologic studies have explored the possible association between parental pesticide exposure and an increased incidence of various birth defects in children. Pages 66-70 of the FDACS report contain descriptions of many published studies in addition to those summarized below.

A literature review by Hanke and Jurewicz in 2004 looked at published studies of pesticide exposure and a number of reproductive outcomes including congenital malformations. The authors conclude that, while the findings are inconsistent, “parental employment in agriculture could increase the risk of congenital malformations in offspring, particularly orofacial cleft. . . as well as defects of musculoskeletal and nervous systems” (Hanke, 2004).

Kristensen, Irgens, et al reported in 1997 that pesticide exposure in Norway, particularly exposure in orchards or greenhouses, was associated with an increased risk of having a child with spina bifida (O.R. =2.76), hydrocephaly (O.R.=2.76), and limb reduction deficits (O.R.=2.5). The exposure measurement was from information provided by men and women identified as farmers from an agricultural census. Birth defects were identified by a national registry. The risk of limb reduction deficits was particularly associated with exposure to pesticides used for grain farming. (Epidemiology 1997 Sept;8(5):537-44).

Garry, Schreinemachers, et. al. examined the frequency of birth defects in children born to pesticide applicators as compared to the general population in Minnesota from 1989-1992. Pesticide use data was derived from data from a survey taken in 1990 by the Minnesota Dept of Agriculture. Counties were clustered by similarity of crops grown and geology. Overall, pesticide applicators had more children born with birth defects in the years of study than the general population (age-adjusted Odds Ratio=1.41). The risk estimates for musculoskeletal anomalies were increased. When stratified by maternal age, however, the risk estimates for women < age 30 were less than 1.0 (i.e. not significant). This study also noted an increased risk of birth defects for pregnancies conceived in the spring, versus the winter or summer. This trend was only found in areas of the state where chlorphenoxy herbicides and fungicides were used, namely western Minnesota where wheat, sugar beets, and potatoes are grown. (Environ Health Perspectives, 1996 104:394-399).

A study conducted in Washington State using state birth records for the years 1980-1993 found ethnicity-adjusted prevalence ratios of 2.6 (C.I.= 1.1-5.8) and 2.6 (C.I. = 0.7-9.5) for limb defects in the children of mothers employed in agriculture compared to children who did not have a parent employed in agriculture and children whose father only was employed in agriculture (Engel, 2000).

A study using birth records from a California hospital found that, within a four-year period, the children of non-agricultural workers had similar rates of major and minor malformations as children having one or both parents in agricultural work. Limb reduction defects, however, occurred more frequently in the children of agricultural workers (5.05 per 1,000 total births versus 2.19 per 1,000 total births) (Schwartz, 1986).

A study in Spain found an increased risk (O.R. = 3.16) for all selected congenital malformations if the mother reported agricultural work or direct handling of pesticides during the period of "acute risk" (defined as one month before conception through the first trimester of pregnancy) as compared to exposure at other times (O.R. = 1.06) (Garcia, 1999).

A recent study of pregnancy outcomes in gardeners and farmers in Denmark found no statistically significant increase in risk estimates for having children with congenital malformations in farmers compared to other workers. The authors note that the results may not be relevant for other countries because, in Denmark, doctors and midwives recommend paid sick leave or change in work tasks if potentially harmful exposures in the workplace are suspected (Zhu, 2006).

More recent epidemiologic studies of pesticide exposure and health outcomes have begun to take advantage of advances in biological measurement of pesticides, their metabolites, or other biological markers of exposure (e.g. cholinesterase levels) to ascertain exposure. Most published epidemiologic studies of pesticide exposure have used surrogate exposure measurements, such as job title, county of residence, or residence in an area where certain crops are grown. Even with the use of biomarkers to ascertain exposure, capturing

exposure at the time in pregnancy when the fetus is most susceptible remains a challenge. Two ongoing studies of interest are the Agricultural Health Study and the CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) study. The Agricultural Health Study is studying the pesticide exposures and health outcomes of pesticide applicators and their families in North Carolina and Iowa (www.aghealth.org). The CHAMACOS study is a cohort study enrolling pregnant women receiving prenatal care at one of two health clinics in the Salinas Valley. One study goal is to investigate the effects of chronic, low-level pesticide exposure on pregnant women and on the growth, health, and neurologic development of their children. The study investigators are using concentrations of pesticide metabolites in subjects' urine as a measure of exposure (www.chamacos.org).

Limitations

The limitations of our assessment include lack of first-hand review of the medical records of two of the children of concern and a lack of knowledge about paternal factors that might increase the risk of a birth defect in their child. What is known about the teratogenic potential of the pesticides of concern is largely derived from animal testing. OEEB's exposure assessment is based on records of worker field assignments and records of pesticide applications that may not be precise. It is not possible to state with complete certainty whether dermal, oral or inhalational exposures to these pesticides occurred, because no biomonitoring or other exposure measurements of the workers were done and environmental data are lacking. These limitations are not unusual in a retrospective assessment of occupational exposures.

Summary

The Occupational and Environmental Epidemiology Branch of the NC Division of Public Health worked with the NC Birth Defects Monitoring Program and the Pesticide Section of the NC Department of Agriculture to assess the potential occupational pesticide exposures during pregnancy of three women employed in farm labor in North Carolina. These women subsequently, unfortunately, gave birth to children with serious birth defects. The following is a summary of the authors' knowledge and conclusions for each of the three case-mothers.

Case-mother 1 worked in North Carolina in 2004 for almost six months; about half of this time period was within the period of concern for her pregnancy. Based on records available, she possibly worked as many as 256 hours within the restricted entry interval for multiple pesticides. During telephone interview, she reported being sprayed with pesticides while working. The evidence suggests that she was exposed to pesticides during the period of gestation when limb development occurs. At least two of the pesticides to which she was possibly exposed have caused limb defects in animal testing. Approximately one third of congenital limb deficiencies occur due to unknown causes. In general, risk factors for having a child with a birth defect include advanced maternal age, a family history of birth defects, a history of a previous pregnancy with birth defects, medication or other drug use, and dietary deficiencies. Heavy alcohol use and smoking have also been associated with limb defects. None of these risk factors have been reported by Florida investigators for Case-mother 1 nor acknowledged by her in

interview. Some epidemiologic studies have reported a higher risk of having children with limb defects in farmworkers with occupational exposure to pesticides as compared to controls without exposure to agricultural pesticides. Given the lack of other known risk factors, the teratogenicity of some of the pesticides in animal studies, the timing of the exposure in relationship to the gestational age of the fetus, and the apparent work environment (multiple violations of pesticide use regulations), there is a plausible association between this mother's possible occupational pesticide exposures in North Carolina and the limb defects seen in her child.

Case-mother 2 also worked in North Carolina during the period of concern for her pregnancy. During her time in North Carolina she possibly entered fields in violation of the REI for a number of pesticides, including one that has been associated with micrognathia in animal testing. This mother also worked in Florida and had at least one day of possible exposure to pesticides within an REI. There is no report of medication or other drug use or dietary deficiencies that would increase the risk of birth defects for this case-mother. The reported micrognathia in the child's father does suggest a possible inherited genetic cause for the occurrence of the birth defect seen in their child. It is possible that an environmental exposure and a genetic susceptibility could have acted together to produce the observed birth defect. Because of the micrognathia in her child's father and the shorter duration of her possible pesticide exposures in North Carolina, the evidence for an association between her pesticide exposure in North Carolina and the birth defect seen in her child is less strong than that for Case-mother 1. A possible contribution of a teratogenic pesticide exposure cannot be ruled out because of the temporal relationship of her possible exposure to multiple pesticides and her birth outcome. A better estimate of pesticide exposure risk for this case-mother is possible if the U.S. EPA or the National Institutes of Occupational Safety and Health (NIOSH) can cumulatively review her possible exposures in both Florida and North Carolina during pregnancy.

Because Case-mother 3 worked in North Carolina only after the period of concern for her pregnancy, her pesticide exposures are not addressed in this report. Her potential exposures as addressed in the Collier County Health Department (CCHD) report. As noted in Table 3 of that report, she worked in fields in Florida on dates corresponding to estimated gestational ages in days of 22 though 34. She did apparently work five days in fields in Florida in early-entry situations when exposure to pesticide residues above levels considered health-protective is likely. This mother gave birth to a female child with multiple birth defects, including some that have been reported in lab animals after pesticide exposure.

Reports from regulatory agency inspections provide important background information on the case-mothers' work environment. The company that employed all three of the case-mothers has been cited by the departments of agriculture in two states for numerous violations of pesticide regulations that include regulations regarding Restricted Entry Intervals, training, disposal of pesticide containers, and decontamination. The women who are the focus of this report may have entered fields prior to the expiration of the REI for multiple pesticides. On a number of days, the case-mothers may have worked in a

field on the date of expiration of an REI. These times were not counted as a possible REI violation in our analysis, however, pesticide residues may still have been present at levels sufficient to cause exposures of concern. In its August 2002 Interim Reregistration Eligibility Decision (IRED), the EPA recommended that the REI for Monitor (methamidophos) used on tomato crops should be increased from 48 hours to 4 days in all states except California. This recommendation has not yet led to a labeling change for Monitor. Ag-Mart was cited by the NC Department of Agriculture for allowing workers to enter fields prior to the expiration of the 48 hour REI for Monitor (as well as other pesticides). The NC Department of Agriculture has also cited Ag-Mart for applying Monitor 4 Spray more often than is permitted during a growing season (Appendix C, p.4). More frequent applications of a pesticide than is allowed could lead to elevated concentrations of that pesticide in foliar residues. Handwashing is a means of preventing or minimizing pesticide exposures. Ag-Mart has been cited for failing to provide adequate handwashing or other decontamination supplies at one of their two farm sites (Appendix C, pp.10-11). Given the numerous cited violations, the over-application of at least one pesticide (Monitor), and the exposure reported by Case-mother 1 in interview, it is probable that at least one of the three case-mothers was unnecessarily exposed to agricultural pesticides, possibly at concentrations above those considered safe.

One argument given against pesticide exposure as a cause of the birth defects in these case-infants is the observation that none of the case-mothers appears to have experienced acute pesticide toxicity. This argument is problematic in two ways. One problem is that these women may not have recognized symptoms of pesticide toxicity. Symptoms of pesticide toxicity such as nausea or headache may have been attributed to pregnancy or other causes (DHHS, 2005). Also, farm worker access to medical care is often limited and, even when care is possible, physicians may not recognize pesticide-related illnesses. In North Carolina, there is currently no routine public health surveillance for pesticide-related illnesses, though efforts to establish this are underway. Highlighting the access to care issue is the fact that none of the three case-mothers received prenatal care prior to the second trimester of pregnancy. A second problem with this argument is that there is evidence from animal studies and human experience that a fetus can be harmed without obvious toxicity in the mother. In one study of dermal exposure of pregnant rats to varying doses of ethylene thiourea (ETU), the dose of 50 mg/kg/day on days 12-13 gestation produced malformations, including missing leg bones and short mandible, in all of the offspring without “any observable significant effects on the dams.” (FDACS report, p. 37). A study of the organophosphate chlorpyrifos found that it was teratogenic in mice when given by intraperitoneal injection at doses below those that caused significant maternal toxicity (Tian, 2005). In historical human experience, methyl mercury is an example of a substance that may harm the developing fetus without producing illness in the mother.

A focus on acute toxicity of pesticide exposure, while important, does not address the risk of repeat exposures to multiple pesticides—the exposure scenario for many agricultural fieldworkers including the women described in this report. Fenske notes that while “on any given day, the pesticide exposure of a fieldworker may be lower than that of a pesticide handler, the frequency of exposure (days per season) may be substantially greater, resulting in a relatively high cumulative exposure” (Fenske, 1997). The EPA has

begun to address this issue with the provision of cumulative risk assessment guidelines for pesticides that have a common mechanism of toxicity (eg. organophosphates).

Another area of uncertainty is the effect of mixtures of chemicals on human health. Most toxicology studies examine the effects of chemicals as isolated agents, yet much human exposure occurs to chemical mixtures. Agricultural pesticides are often applied to fields as mixtures. Such mixtures could be anticipated to have additive, synergistic, or inhibitory effects on toxicity. One study of neurotoxicity found that mice exposed to the pesticides paraquat and maneb individually and in combination showed no effect of the individual chemicals at the doses given, but significant effects when the doses were combined (Cory-Slechta, 2004). A study of the embryonic toxicity of Dithane M45 (80% mancozeb) and copper sulphate given by injection to pheasant egg chambers found that the simultaneous administration of the compounds caused higher toxicity (93% embryo mortality) than either copper sulphate (68% mortality) or Dithane M45 (50% mortality) alone (Szabo, 2003).

Conclusions

This review of available North Carolina exposure data indicates a plausible association between possible pesticide exposure and the limb deficiencies seen in Case-mother 1's child. An association between possible pesticide exposures in North Carolina and the jaw and palate abnormalities seen in Case-mother 2's child cannot be ruled out; however, there is evidence to suggest familial inheritance. Case-mother 3 worked in North Carolina only after the period of concern for her pregnancy--it is therefore extremely unlikely that any occupational pesticide exposures that may have occurred in North Carolina could have been associated with the multiple severe defects seen in her child. Case-mothers 2 and 3 also had potential pesticide exposures in Florida during the period of concern for their pregnancies. It cannot be determined with certainty whether maternal pesticide exposure caused birth defects in any of the case-infants because of the small number of cases, the lack of complete information on exposure dosage, and other variables. While it is possible that the birth defects are unrelated to the case-mothers' occupational exposures, there is evidence, based on interview information and regulatory compliance information from the NC Departments of Agriculture and Labor, that the women's work environment likely put them at an increased risk of over-exposure to pesticides. In conclusion, the findings of this investigation warrant concern and action on the part of public health and regulatory agencies charged with protecting the health of farmworkers.

Recommendations:

1. Request that NIOSH and/or U.S. EPA study the aggregate potential exposures of the case-mothers, particularly Case-mother 2, in Florida and North Carolina.

2. North Carolina state government agencies including the Department of Environment and Natural Resources, the Department of Agriculture and Consumer Services, and the Department of Labor and the Division of Public Health should collaborate to ensure that programs are in place to provide consistent protection of farmworkers from exposure to pesticides. This

collaboration should include sharing information on violations of pesticide regulations and worker protection violations.

3. Establish a working group comprised of representatives from public health, agriculture, migrant health, and advocacy groups working with farmworkers in North Carolina to assess current prevention and enforcement efforts relating to pesticide safety. This has been initiated by public health. The goal of this initiative is to strengthen efforts to educate farmworkers about their rights under the Worker Protection Standard, to develop pesticide education materials targeting women of childbearing age, and to educate physicians in North Carolina about including occupational exposures in their history taking, especially during prenatal care.

4. Expand the North Carolina Department of Agriculture and Consumer Services' current on-farm compliance monitoring and enforcement related to pesticide handling and use and agricultural worker protection initiatives.

5. Establish a public health surveillance program for acute pesticide-related illnesses in North Carolina. OEEB has begun this process and has applied for funding. A surveillance system will help improve our state's ability to detect and respond to cases of reported pesticide-associated illness right away. A rule requiring physician reporting of acute pesticide-related illnesses has been approved.

6. Explore the feasibility of adding parental occupational information to NC birth certificate data. This would provide occupational data on all birth defects cases and allow comparisons of rates of birth defects between different occupational groups.

7. Recommend to the U.S. EPA that the requirements of the Worker Protection Standard be strengthened to require more specific documentation of workers' field locations, field entry times, and pesticide application times. This would improve investigations of REI compliance.

Appendices:

Appendix A: FDACS report
Appendix B: CCHD report
Appendix C: NC Notice of Violations
Appendix D: Restricted Entry Interval Requirements for Pesticides in Text Tables

References:

Chapin RE, Robbins WA, Schieve LA, et.al. Off to a Good Start: The Influence of Pre- and Periconceptional Exposures, Parental Fertility, and Nutrition on Children's Health *Environmental Health Perspectives* 112: No. 1 p.3 (2004).

Coye MJ and Fenske R Agricultural Workers in Levy and Wegman (Eds.): Occupational Health – Recognizing and Preventing Work-Related Disease. Boston. Little, Brown & Co., 1988, Chapter 33.

Department of Health and Human Services (DHHS/CDC/NIOSH). Pesticide-related illness and injury surveillance. 2005. DHHS-NIOSH Publication No. 2006-102, p. 5.

Engel LS, O'Meara ES, Schwartz SM. Maternal occupation in agriculture and risk of limb defects in Washington State, 1980-1993. *Scand. J. Work Environ. Health*. 2000 June; 26 (3): 193-8.

Fenske, Richard. Pesticide Exposure Assessment of Workers and their Families In Keifer, MC (Ed.): Human Health Effects of Pesticides. Occupational Medicine State of the Art Reviews. Vol. 12, No. 2, April-June 1997. Philadelphia, Hanley & Belfus, Inc.

Food and Drug Administration online patient information on thalidomide: http://www.fda.gov/cder/news/thalidomide.htm

Garcia AM, Fletcher T, Benavides FG, Orts E. Parental Agricultural Work and Selected Congenital Malformations. *American Journal of Epidemiology* 1999; 149:64-74.

Hanke, W. and Jurewicz, J. The Risk of Adverse Reproductive and Developmental Disorders Due to Occupational Pesticide Exposure: An Overview of Current Epidemiological Evidence. *International Journal of Occupational Medicine and Environmental Health*, 2004; 17(2):223-243.

Holmes, LB. Teratogen-Induced Limb Defects. American Journal of Medical Genetics 2002; 112: 297-303.

Improved National Prevalence Estimates for 18 Selected Major Birth Defects---United States, 1999-2001. *MMWR*. Jan. 6, 2006. 54 (51&52); 1301-1305.

Jakobsen LP, Kundsen MA, Lespinasse J, et al. The Genetic Basis of the Pierre Robin Sequence. *The Cleft Palate-Craniofacial Journal* 2005; 43 (2) [abstract].

Makhoul IR, Goldstein I, Smolkin T, et al. Congenital limb deficiencies in newborn infants: prevalence, characteristics and prenatal diagnosis. *Prenatal Diagnosis* 2003; 23:198-200.

NC Department of Labor, Occupational Safety and Health Administration (OSHA) Ag-Mart Produce Inc. Inspection No. 306923095; 306923103; 306923111. http://www.osha.gov/pls/imis/establishment.inspection.html Open date 8/21/2003.

Nurminen, T. The Epidemiologic Study of Birth Defects and Pesticides. Epidemiology March 2001, Vol 12 No. 2 p. 145.

Schwartz DA, Newsum LA, Heifetz RM. Parental occupation and birth outcome in an agricultural community *Scand J of Work Environ Health* 1986 Feb; 12 (1):51-4.

Smith JL, Garry VF, Rademaker AW and Martin RH. Human Sperm Aneuploidy After Exposure to Pesticides. *Molecular Reproduction and Development* 2004. 67:353-359.

Stal S, Hollier LH, Edwards M. Syndromes with craniofacial abnormalities. In UpToDate: www.uptodate.com written July 2004, downloaded 2/6/06.

Stevenson RE, Hall JG, Goodman RM (Eds), Human Malformations and Related Anomalies, Vol II., part of Oxford Monographs on Medical Genetics No. 27, Oxford University Press, 1993, pp.703-715.

Tian Y, Ishikawa H, Yamaguchi T, Yamauchi T, Yokoyama K. Teratogenicity and developmental toxicity of chlorpyrifos: Maternal exposure during organogenesis in mice. *Reproductive Toxicology* 2005; 20: 267-271.

U.S. Environmental Protection Agency, Mancozeb: Health Effects Division Human Health Risk Assessment to Support Reregistration, Washington, DC, June 3, 2005.

U.S. Environmental Protection Agency Methamidophos Interim Re-registration Eligibility Decision http://www.epa.gov/REDs/factsheets/methamidophos_ired_fs.htm

Worker Protection Standard – How to Comply Manual as Revised 2005. www.epa.gov

Zhu JL, Hjollund NH, Andersen AN, Olsen J. Occupational Exposure to Pesticides and Pregnancy Outcomes in Gardeners and Farmers: A Study Within the Danish National Birth Cohort. *Journal of Occupational and Environmental Medicine* 2006;48:347-352.