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Occupational exposure to pesticides and risk of leukemia among offspring in Costa Rica

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To Jose, Tati, Pauli and Fiore

ABSTRACT

Background. Leukemias are the most common childhood cancers, accounting for 25-35% of the incidence of all childhood cancer in most populations. In early reports on cancer, Costa Rica ranks among the highest incidence of childhood leukemia in the world. Agriculture is an important economic activity in Costa Rica and is characterized by intensive use of pesticides. In this thesis we present a descriptive epidemiologic study of childhood leukemias, two methodological studies on parental pesticide exposure assessment and an etiological analysis based on a population based-control study.

Objective. The main objective of this study was to increase the understanding of potential risk factors of childhood leukemia in Costa Rica, propose improved methods for exposure assessment in cancer research in developing country conditions, and provide epidemiological evidence of association between childhood leukemia and parental occupational pesticide exposure during the prenatal and early postnatal periods of children.

Methods. Childhood leukemia incidence during 1981-1996 was analyzed by histology, gender, birth year, time period of diagnosis, age at diagnosis and region. Case data were extracted from the National Cancer Registry of Costa Rica. Person-years at risk were calculated from census data and annual sample survey data. International comparison of rates was performed (Paper I). We describe an icon-calendar interview form (ICF) for assessing retrospective parental exposure to pesticides, collecting data on agricultural pesticide use, job tasks, frequency of exposure and major determinants of pesticide exposure (Paper II). ICF data were combined with external data of pesticide application rates for the country, in two retrospective exposure assessment models (Paper III). Associations between parental exposure to pesticides and the risk of leukemia in the offspring were examined in a population-based case-control study in Costa Rica. All cases of childhood leukemia (N=334), ages 0-14 at diagnosis, during 1995-2000, were identified at the Cancer Registry and the Children's Hospital of Costa Rica (ICD-0-1). Population controls (N=579), were drawn from the National Birth Registry (Paper IV).

Results. The reported cases of childhood leukemia between 1981-1996 represented an overall age-standardized incidence rate of 55 per million person-years. Acute lymphocytic leukemia (ALL) accounted for 79% of all leukemias. A Poisson regression model identified significant excesses of ALL for boys, groups of 1-4 and 5-9 years of age and for three out of seven geographical regions. Based on the first 100 ICF, recall was acceptable in 90% of the interviews, successful identification of special events in 84%, good recovery of pesticide names, frequency of use and application techniques in 90%, report of changes in pesticide use patterns in 63%. Numbers of exposed mothers and fathers for each prioritized pesticide and its exposure intensities for five time windows, were estimated with the exposure assessment models. A sensitivity analysis suggested satisfactory robustness of the model weights. In the case-control

study, 876 mothers and 762 fathers were included in the analysis. Mothers' exposures to any pesticides anytime and during year before conception and 1st and 2nd trimesters, and fathers' exposures to any pesticides during 2nd trimester were associated with excess risks. Excesses of total leukemia were found for mothers and fathers exposed to organophosphates and for fathers exposed to benzimidazoles. There was a suggestion of exposure-response gradients for fathers for picloram, benomyl and paraquat. Age at diagnosis was positively associated with fathers' exposures and inversely with mothers'. Mothers' exposures seemed more important than fathers'.

Conclusions. Based on worldwide epidemiologic data, including ours, we believe that parental and early childhood exposure to pesticides may be associated with risk of leukemia in the offspring. The most likely candidates for etiologically important pesticidal compounds appear to be organophosphates as group and – still with considerable uncertainty – picloram, benomyl, paraquat, foxim, mancozeb, malathion, dichlorvos and sodium monomethyldithiocarbamate (metam sodium). Epidemiological research on childhood cancer needs to be further conducted in developing countries, including etiological research on pesticides. There is a need to study biomarkers for assessing biological processes in leukaemogenesis, susceptibility and exposure. We recommend prevention of pesticide hazards, especially in the population stratum reproductively active and among children.

Key words*: agriculture, agricultural workers, cancer epidemiology, case-control, childhood cancer, childhood leukemia, children, Costa Rica, developing countries, exposure assessment, fetal exposures, offspring, prenatal exposures, icons, interview, occupational exposure, pesticides, questionnaire, pregnancy, reproductive effects, tropics.*

RESUMEN

Antecedentes. Las leukemias son las enfermedades malignas más frecuentes en la infancia y representan entre un 25-35% de la incidencia total de cáncer infantil en la mayoría de la poblaciones. La incidencia de leucemia en niños en Costa Rica, se encuentra entre las más altas en el mundo. La agricultura es una actividad económica muy importante en el país, caracterizada por el uso intensivo de plaguicidas. En esta tesis se presentan un estudio descriptivo epidemiológico de leucemias en niños, dos estudios metodológicos sobre evaluación de exposición de padres a plaguicidas y un estudio analítico de casos y controles de base poblacional sobre la asociación entre la exposición paterna ocupacional a plaguicidas y el riesgo de leucemia en los hijos.

Objetivo. El objetivo principal del estudio fue incrementar el conocimiento de los factores de riesgo asociados al desarrollo de leucemias infantiles en Costa Rica, proponer métodos de evaluación de exposición en cáncer para países en desarrollo y evaluar la asociación entre las leucemias en niños y la exposición paterna ocupacional a plaguicidas durante el período prenatal y postnatal temprano.

Métodos. La incidencia de leucemia en niños durante el período 1981-1996 fue analizado por histología, sexo, año de nacimiento, año de diagnóstico, edad al diagnóstico y región geográfica. La información de los casos fue extraída del Registro Nacional de Tumores (RNT). Los años-persona en riesgo fueron calculados del censo y de las estimaciones de población para estos años. Se establecieron comparaciones internacionales de incidencias (Artículo I). Presentamos un cuestionario pictórico (calendario de imágenes) (ICF) para evaluar la exposición retrospectiva de exposición a plaguicidas de ambos padres, que se utilizó para recopilar la información sobre uso de plaguicidas en agricultura, puestos de trabajo y los principales determinantes de exposición (Artículo II). Los datos obtenidos con el ICF fueron combinados con datos externos de tasas de aplicación de plaguicidas, en dos modelos de evaluación de exposición retrospectiva (Artículo III). Adicionalmente se analizó la asociación entre la exposición paterna y materna a plaguicidas y el riesgo de desarrollar leucemia en los niños, a través de un estudio de casos y controles. Todos los casos de leucemia (N=334) con edades entre 0-14 años en al momento del diagnóstico, durante 1995-2000 fueron identificados en el RNT y el Hospital Nacional de Niños de Costa Rica. Los controles fueron escogidos del Registro Civil (Manuscrito IV).

Resultados. Los casos reportados de leucemia infantil en el período 1981-1996 representaron una incidencia estandarizada por edad de 55 por millón de años-persona. El 79% correspondió a leucemia linfocítica aguda (LLA). Un modelo de regresión de Poisson identificó excesos significativos de LLA para niños, los grupos de edad 1-4 y 5-9 y para tres de las siete regiones geográficas. Con base en los primeros ICF completados: la capacidad de recordar fue aceptable en 90% de las entrevistas, la identificación exitosa de los eventos especiales en

un 84%, la recopilación general de uso de plaguicidas en un 90%, y el reporte de cambios en los patrones de uso de plaguicidas en un 63%. Los modelos de evaluación de exposición identificaron la cantidad de madres y padres expuestos a los plaguicidas priorizados y sus intensidades de exposición para 5 ventanas de tiempo. Un análisis de sensibilidad del modelo cuantitativo sugirió una adecuada fuerza de los pesos utilizados. En el estudio de casos y controles, 876 madres y 762 padres fueron incluidos en el análisis. La exposición de las madres a plaguicidas en general, durante el año antes de la concepción, 1º y 2º trimestres de embarazo y durante todo el período fue asociada con el riesgo de leucemia infantil. También se identificó riesgo para los padres expuestos a plaguicidas en general, durante el 2º trimestre de embarazo del niño. Se identificaron asociaciones en madres y padres expuestos a organofosforados y riesgos de leucemia, así como padres expuestos a benzimidazoles. Es posible que hubiera una gradiente de exposición-respuesta para padres expuestos a picloram, benomil y paraquat. La edad del niño al diagnóstico estuvo asociada positivamente con los padres y en forma inversa con las madres. Las exposiciones maternas parecen más importantes que las de los padres.

Conclusiones. En base a los datos epidemiológicos internacionales, incluidos los nuestros, consideramos que hay razones que apoyan la asociación entre la exposición paterna a plaguicidas pre-concepción y temprana en la niñez y el riesgo de leucemia infantil. Los plaguicidas que podrían ser agentes etiológicos son organosforados como grupo y -aún con alguna incertidumbre- benomil, paraquat, picloram, foxim, mancozeb, malatión, diclorvos y metilditiocarbamato sódico. Investigaciones epidemiológicas en cáncer infantil son necesarias en países en desarrollo, incluyendo la investigación etiológica en plaguicidas. Es importante el desarrollo de biomarcadores para evaluar los procesos biológicos de leuquemogénesis, susceptibilidad y exposición en Costa Rica. Recomendamos la prevención de riesgos asociados con plaguicidas, especialmente en la población en edad reproductiva y en los niños.

Palabras clave*: agricultura, cáncer infantil, casos y controles, Costa Rica, cuestionario embarazo, efectos reproductivos, epidemiología de cáncer, entrevista, evaluación de exposición, exposiciones fetales, exposición ocupacional, íconos, leucemia, niños, países en desarrollo, plaguicidas, trabajador agrícola, trópico.*

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numeral (I-IV):

I. Monge P, Wesseling C, Rodríguez AC, Cantor K, Weiderpass E, Reutfors J, Ahlbom A, Partanen T. Childhood leukemia in Costa Rica, 1981-1996. Paediatr Perinat Epidemiol 2002;16:210-18.

II. Monge P, Wesseling C, Engel L, Keifer M, Zuurbier M, Rojas M, Partanen T. An icon-based interview for the assessment of occupational pesticide exposure in a case-control study of childhood leukemia. Int J Occup Environ Health 2004;10:72-78.

III. Monge P, Partanen T, Wesseling C, Bravo V, Ruepert C, Burstyn I. Assessment of pesticide exposure assessment in the agricultural population of Costa Rica. Ann Occup Hyg 2005;49:1-10.

IV. Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor K, Weiderpass E, Partanen T. Parental occupational exposure to pesticides and risk of childhood leukemia in Costa Rica. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

a.i.	Active ingredient
ALL	Acute lymphocytic leukemia
ANLL	Acute non-lymphocytic leukemia
CCSS	Social Security System of Costa Rica *(Caja Costarricense de Seguro Social)*
CI	Confidence interval
DDT	Dichloro-diphenyl-trichloroethane
DBCP	Dibromochloropropane
EPA	U.S. Environmental Protection Agency
HNN	National Children's Hospital *(Hospital Nacional de Niños)*
IARC	International Agency for Research on Cancer
ICCC	International Classification of Childhood Cancer
ICD-O	International Classification of Diseases for Oncology
ICF	Icon-calendar form
INEC	National Institute of Statistics and Censuses *(Instituto Nacional de Estadística y Censo)*
IRET-UNA	Central American Institute for Studies on Toxic Substances *(Instituto Regional de Estudios en Sustancias Tóxicas, Universidad Nacional, Heredia, Costa Rica)*
NA	Not applicable
NEC	Number of exposed cases
NOS	Not otherwise specified
OR	Odds ratio
PPP	Purchasing power parity
RNT	National Cancer Registry *(Registro Nacional de Tumores)*

1 INTRODUCTION

Childhood leukemias are the most common cancers among children, accounting for 25-35% of the incidence of all childhood cancers in most populations (Little 1999, Parkin 1998). The etiology of childhood leukemia is still to a large extent unknown. A number of risk factors have been proposed in the literature, such as gender, age, genetic factors, ethnicity, socio-economic status, high birth weight, exposure to ionizing radiation, spores, infections, dust, fumes, and chemical agents.

Epidemiological cancer data from developing countries are scarce and much needed. During the 1980s, the newly established cancer registry in Costa Rica permitted the identification of childhood leukemia as the most common childhood malignancy (39% in 1987), with incidence rates among the highest in the world: 59 per million for total leukemia, and 45 per million for acute lymphocytic leukemia in 1987 (Little 1999, Parkin et al. 1998, Linet et al. 1991, Sierra et al. 1995). Associations of childhood leukemia with parents' exposure to pesticides were proposed in medical discussions, and a cluster associated with pesticide exposure in the late 1980s caused considerable public concern, but no formal investigations were possible at that time.

An increased risk of leukemia in children associated with parental occupational exposure to pesticides prior and during pregnancy has been suggested by a number of epidemiologic studies (Shu et al. 1988, Infante-Rivard et al. 1999, Petridou et al. 2000, Meinert et al. 2000, Alexander et al. 2005). Agriculture is an important economic activity in Costa Rica and is characterized by intensive and inappropriate use of pesticides, many of them mutagenic, genotoxic and possibly carcinogenic (Wesseling et al. 2001). An epidemiologic study examining the relation between parental pesticide exposures and cancer in the offspring emerged therefore as a priority, in spite of challenges to carry out cancer epidemiology in a developing country.

In this thesis we present a descriptive epidemiologic study of childhood leukemias, two methodological studies on parental pesticide exposure assessment, and an etiologic analysis based on a population-based case-control study.

2 BACKGROUND

2.1 Leukemias

Leukemias are a group of heterogeneous neoplastic disorders of white blood cells, a clonal disorder of stem cell, primary at the bone marrow. Leukemic cells usually spill into the blood, and may also infiltrate lymph nodes, liver, spleen and other tissues. Leukemias can be subdivided into lymphoid (originating from a precursor of B- or T- lymphocytes) and myeloid (originating from a precursor of granulocytes, monocytes, erythrocytes, or megakaryocytes). According to the International Classification of Childhood Cancer (ICCC), adapted from the International Classification of Diseases (ICD-0-3), leukemias are classified into five groups: lymphoid leukemias, acute myeloid leukemias, chronic myeloproliferative diseases, myelodysplastic syndrome and other myeloproliferative diseases, and unspecified and other specified leukemias. Table 1 shows the classification (Steliarova-Foucher et al. 2005).

Table 1. International classification of childhood leukemias[1].

Diagnostic groups	Morphology
Ia: Lymphoid leukemia	9820, 9823, 9826, 9827, 9831-9837, 9940, 9948
Ib: Acute myeloid leukemias	9840, 9861, 9866, 9867, 9870-9874, 9891, 9895-9897, 9910, 9920, 9931
Ic: Chronic myeloproliferative diseases	9863, 9875, 9876, 9950, 9960-9964
Id: Myelodysplastic syndrome and other myeloproliferative diseases	9945, 9946, 9975, 9980, 9982-9987, 9989
Ie: Unspecified leukemia	9800, 9801, 9805, 9860, 9930

1- Based on the International Classification of Childhood Cancer (ICCC-3). Steliarova-Foucher E, Stiller, C, Lacour B, Kaatshc P. International Classification on Childhood Cancer, Third Edition. International Agency for Research on Cancer (IARC). Cancer 2005;7:1457-67.

2.1.1 Epidemiology of childhood leukemias

Leukemias are the most common childhood cancers, especially among white populations in Europe, America, Oceania and in most of Eastern Asia, with an age-standardized rate of 35-50 per million. Acute lymphocytic leukemia (ALL) comprises around 80% of the total leukemias in most populations (Little 1999, Parkin 1998). The highest incidence have been reported in Hispanic populations of California (Glazer et al. 1999), Florida (Wilkinson et al. 2001), and Costa Rica (Linet 1991).

The etiology of childhood leukemia is not well understood. Known or suspected risk factors include gender, age, genetic factors, ethnicity, socio-economic status, small families; parental, child and prenatal exposure to ionizing radiation, electromagnetic fields, chemical agents, dusts, fumes, medications, infections, and other events in the medical history of parents and the child (Little 1999, Ries et al. 1999).

2.1.2 Occupational and environmental exposures and childhood leukemias

Exposures to occupational and environmental hazards may contribute to the risk of leukemia in offspring through different mechanisms: intrauterine and early extrauterine exposure via maternal or paternal route of exposure to toxic compounds. Metabolites of parental exposures have been proposed through transplacental transmission during gestation. Children may be exposed during the postnatal period by take-home exposure through contaminated person or work clothes (Savitz et al. 1990, Ries et al. 1999, Curl et al. 2002, Buffler et al. 2005). Environmental exposures during development and childhood also may place children at higher cancer risk than adults at the same level of external exposure (NRC 1993, van Larebeke et al. 2005). Differences in anatomy and physiology between child and adult may influence the risk associated with chemical exposure.

2.2 Costa Rica

2.2.1 Population, health indicators and health care

Costa Rica is a tropical country located in Central America, with both Atlantic and Pacific Ocean coastlines. It has an area of 51,100 km^2 and purchasing power parity (PPP) (adjusted per capita estimation among countries) of $9,600/year. Table 2 shows some population and health indicators for the years 1995, 2000 and 2005.

Table 2. Population and health indicators in Costa Rica, 1995, 2000 and 2005.

	1995	2000	2005
Population (millions)	3.3	3.9	4.2
Number of births	80,306	78,178	71,548
Birth rate (per 1000 inhabitants)	24.3	20.0	17.0
Infant mortality rate (per million)	13.2	10.2	9.2
Life expectancy (years)	76.2	77.7	78.7

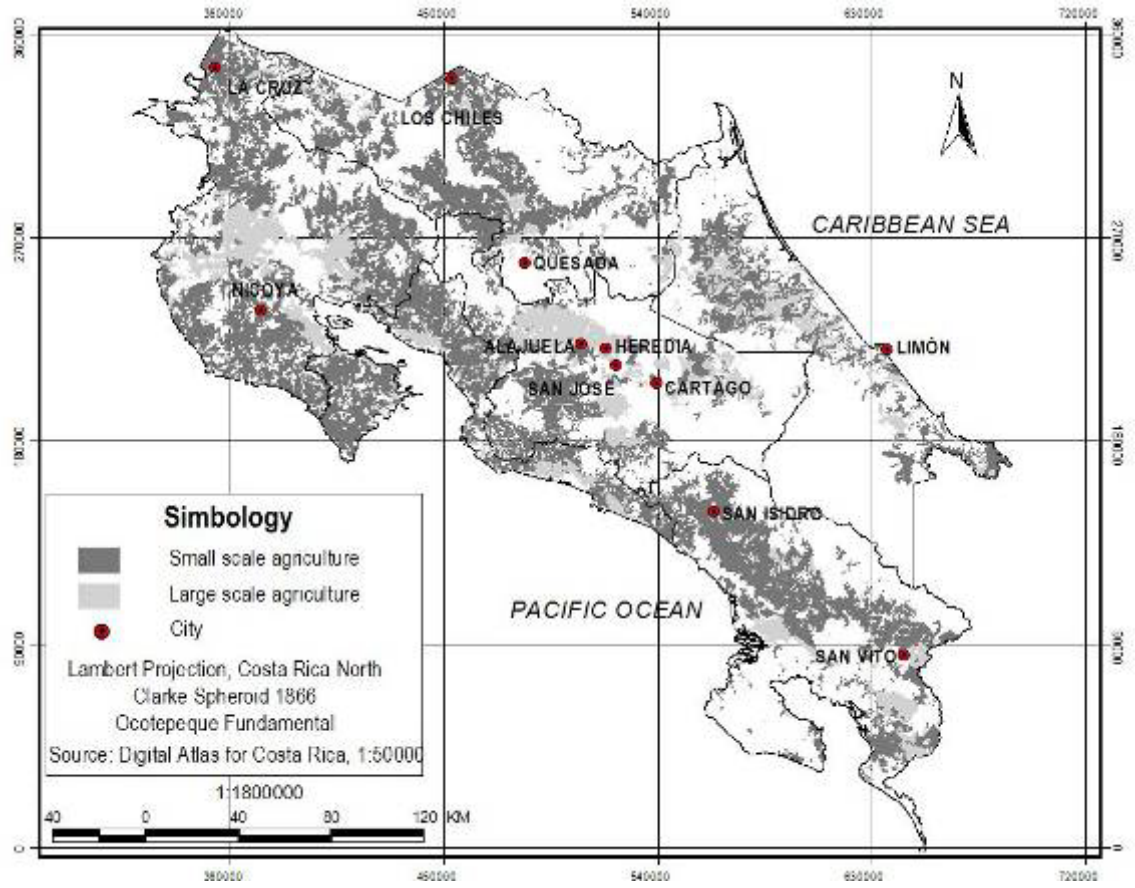
Source: Annual Statistics htttp//: www.inec.gov.cr

Costa Rica has a satisfactory health care system and reasonably good population and disease registries, including a civil registry with a unique personal identification number and legally mandated cancer registration. The Social

Security System of Costa Rica (CCSS) offers medical assistance for 81% of the total population (http://www.info.ccss.sa.cr/). The population is ethnically homogeneous, most Costa Ricans being mestizos (caucasian-indigenous) as a result of four centuries of racial mixing. The indigenous, black and Chinese populations count for approximately 1, 2 and 1% of the total population, respectively. A National Cancer Registry (Registro Nacional de Tumores, RNT) was created in 1976, and its coverage became nationwide in 1980.

The agricultural regions and principal population centers of Costa Rica are shown in Figure 1.

Figure 1. Agricultural regions and principal population centers, Costa Rica.



2.2.2 Registration of leukemia cases

Notification to the RNT of each diagnosed cancer case is mandatory for all hospitals and clinics (both inpatient and outpatient), as well as for clinical and pathology laboratories (both public and private). Since 1980, RNT has received results of all biopsies with histology reports of cancer and also tumors diagnosed with other methods. In addition, the Registry reviews death certificates at the Central Bureau of Statistics and Census (INEC) and includes data on each cancer death in the RNT database.

The case records at the RNT database include patient name, personal identification number, diagnosing facility, age, gender, place of birth, place of residence, method of diagnosis, primary site of cancer, date of first diagnosis, histologic type, and date of death. Notifications are reviewed for completeness and consistency and, if necessary, checked against original hospital records by technicians trained in medical registration. A computer program updates the case data and removes duplications (Rodríguez 1992, Parkin 2002). Data collected since 1980 have been included in the International Agency for Research on Cancer (IARC) publications of "Cancer Incidence in Five Continents" (Muir 1987, Parkin 1992, Parkin 1997, Parkin 2002). IARC evaluation of the RNT data and inclusion in IARC documents indicate a reasonably high quality of registration and reporting.

Diagnosis of leukemia cases is centralized in the National Children's Hospital (HNN), and occurs occasionally in a few private hospitals. Treatment is almost always carried out at HNN. In 1996, 98% of leukemia cases in children less than 15 years of age, were reported to the RNT by the Hematological Department of HNN, diagnosed and classified primarily by bone marrow smears.

2.2.3 Pesticide use in Costa Rica

Agriculture represents an important economic activity in Costa Rica, although the economically active agricultural population has decreased considerably over the last decade: from 30% in 1984 to 14.3 % in 2004 (INEC 2004). Agriculture in Costa Rica, like in other developing countries, depends on high chemical inputs, associated with excessive use of pesticides (Wesseling et al. 2001). Approximately 348 active ingredients (a.i.) of pesticides were imported in Costa Rica between 1977 and 2002, which represented a total of 140 million kg of a.i. An estimated annual average percentage increase was 9% of the total weight of a.i. for the entire period (de la Cruz et al. 2004). An annual average of 2.5 kg of active pesticide ingredient per inhabitant was estimated for the year 1996, compared, eg, with 0.7 kg in the Netherlands, which represents a high European rate (Chaverri et al. 2002).

Before the 1980s imports were mainly organochlorines (DDT, aldrin, dieldrin, lindane and chlordane among others), but in the early 1980s agricultural use of most organochlorine compounds was banned, and more organophosphorous

compounds were imported. Banning and restrictions were also applied during the 1980s to dibromochloropropane (DBCP), a nematocide responsible for male infertility; the herbicides 2,4,5-T, nitrophen and dinoseb, and the fungicide captafol – all at least possibly carcinogenic by IARC or U.S. Environmental Protection Agency (EPA) (Tables 3 and 7). During the 1990s the use of lead arsenate compounds, endrin, chlordane, heptachlor, lindane and dechlordane were banned for environmental persistence, acute toxicity and carcinogenicity. However, some other highly toxic pesticides are still in use in Costa Rica. Some of them have been banned in other countries such as paraquat (a bipyridyl herbicide), endosulfan (an organochlorine insecticide) and mancozeb (a dithiocarbamate fungicide) (de la Cruz et al. 2004).

The number of carcinogenic pesticides imported to Costa Rica has changed over time. Two million kg of a.i. of pesticides classified as class A, B1 and B2 (U.S. Environmental Protection Agency, EPA) were imported in 1985, while this figure increased to 3 million in 2000. The carcinogenic pesticides imported (using the EPA classification) by highest volumes in those groups are mancozeb and methylarsonic acid (de la Cruz et al. 2004). The numbers of workers occupationally exposed to common pesticides in 2002 varied between 11,000 and 175,000 depending on compound (Partanen et al. 2003).

2.3 Methodological issues in research in childhood leukemia and pesticides

2.3.1 Exposure assessment

Assessment of past exposures is fundamental in occupational epidemiology (Espinosa et al. 2005, Stewart & Stenzel 2000). Generally, for analytic study design (cohort or case-control), information on the job title, type of industry, time of exposure (duration in the job task, frequency of exposure and intensity) and sometimes other related data, are collected for each study subject (Stewart et al. 2001). For the study of past exposures in association to cancer, latency periods must be incorporated in the assessment by lagging exposures.

For past external exposures of which measurements are not available, exposure data ascertainment usually relies on interviews, questionnaires, medical records or vital statistics, and sometimes on agent metabolite bioconcentrations, often long after the etiologically pertinent external exposure took place.

The health of agricultural workers and their families, as related to pesticide exposure, is of public and occupational concern. Assessment of exposures requires different techniques than in studies of industrial workers. While not unique to agricultural populations and their exposures, the highly variable, multifaceted nature of these exposures makes the development of meaningful exposure metrics difficult. Agricultural workers' exposures are influenced by a number of factors, some of which are usually not known at the individual level (weather conditions, clothing, physical activity, variety of job tasks, worker's

knowledge about occupational hazards and exposure to pesticides, among others). The usefulness of published literature on pesticide exposure measurements is limited due to the lack of consistent measurement strategies (Stewart et al. 2001) and incomplete adaptability of those measurements in environments that vary in job settings in different countries, districts and socioeconomic settings (Aragón et al. 2006, Blanco et al. 2005). In addition, in less developed countries several factors contribute to high exposures: pesticide misuse, lack of regulations, lower literacy and a pressure for growing food ingredients (Alexander et al. 2005).

Timing of exposures is important, although its valid estimation is a challenge. Pre and post-natal time windows of exposures by either parent has been used in some studies (Van Steensel-Mol et al. 1985, Meinert et al. 1996, Meinert et al. 2000, Menegaux et al. 2006)

Pesticides are highly heterogeneous groups of chemicals; human exposures are often multiple, and exposure patterns may change rapidly over time. The variety of exposure routes and kinetic-metabolic pathways contributes to the need for complex exposure assessment methods.

A health-based prioritization system for pesticides was developed and used as a component for the exposure assessment of our study. It yielded a list of highest priority pesticides, which were then linked to a historical pesticide use database to produce short lists of priority pesticides (checklists), specific to crops and time periods. After final validation of these lists by crop experts, they were used in the application of the questionnaires to improve recall among the study participants (Section 4). Pesticide import data between 1977 and 2000 were used as surrogates for pesticide usage data (Valcke et al. 2004). Sixty-seven highly prioritized pesticides were identified. Finally, we selected 25 pesticides for the exposure assessment of our study, based on the frequency of occurrence in the data reported in the ICF.

The three major general groups of methods of assessment of past exposures are: exposure matrices for general populations and specific jobs or industries, expert assessment, and exposure assessment models. Each one has its strengths and limitations, and models may include all three of them.

2.3.2 Exposure models

Exposure assessment models are methods for predicting or estimating exposures from determinants of exposure. Determinant data can be collected from primary (self report, interview or questionnaire) or secondary sources (registries or databases). Different sources may be combined in the model (Espinosa et al. 2005).

In chronic disease epidemiology, assessment of exposure to agricultural pesticides has been usually based on surrogates of exposure such as type of farm operation, chemicals used, job titles and duration of employment, but also

on biological samples, the latter usually suffering from the late timing of the sampling, relative to exposure. Since it is unlikely that historical exposure monitoring data will be available, particularly in developing countries, there is a need for constructing models for exposure assessment, which incorporate the most important exposure determinants and modifiers to estimate intensity of pesticide exposure (Dosemeci et al. 2002).

2.3.3 Data sources on pesticide use

Farm owners depend on their crop production for living. Agrochemical use is an important monetary input into agricultural activity in most agricultural settings. Different data sources are available in different countries and localities. In Costa Rica, bank loans are available for agricultural purposes, which, once granted, are monitored by agricultural experts. Written guidelines for technical procedures have been distributed, which include the use of pesticides (concentrations, frequency of use) per crop for the past 25 years. In addition, the Ministry of Agriculture keeps records of all pesticides imported in the country. A database of pesticide imports over the past 30 years has been set up at the Universidad Nacional. Many agronomy students have performed studies about pesticide use in specific crops. Associations of producers (coffee, sugarcane, etc.) also keep records of recommendations of their experts. Based on these different data sources, a matrix was produced with data of pesticide use by time period, crops and regions (Wesseling & Bravo 2002).

2.3.4 Epidemiological research in a developing country

Research in occupational epidemiology is an important challenge in developing countries. Training of personnel, equipment and supplies and the development and implementation of information systems are the bases for this activity. High quality research is needed, for the identification of health problems, assessment of prevention or interventions, and constructing and using feasible methods and available resources (Wesseling et al. 1997, Levy et al. 1992).

Costa Rica offers reasonably good conditions for epidemiological studies of cancer. As mentioned above, it is one of the few developing countries with a satisfactory health care system, reliable population and disease registries, including a civil registry with unique personal identification number, and a national population-based cancer registry. But there are other aspects that make research more complex as compared with developed nations. For example, in the civil registry, addresses are recorded for the place of birth only and often in a very scanty way; occupations, although included in the 2000 census data, are not provided for each individual in any of the registries, nor are telephone numbers included. Therefore procedures for individual identification of participants need creative extra efforts. Our strategy for data collection of the study required personal interviews through visits to the participants' homes. The procedure for locating the addresses was time consuming and meant sometimes driving long distances, for 4 to 6 hours, and walking to faraway locations for several hours. Originally the field work was planned for 18 months, but it took 42 months to finish it.

Summarizing, given the high rate of childhood leukemia in Costa Rica and high pesticide use, the previous studies associating childhood leukemia and pesticide use in parents, the existence of a reasonably good cancer registry, indirect exposure data and epidemiology expertise, it was felt justified to perform a study exploring the associations between parental occupational exposure to pesticides and childhood leukemia in the offspring.

Research assistant walking to a participant's home. Picture taken by Rocío Loría

3 OBJECTIVES

- To describe incidence of childhood leukemia in Costa Rica in relation with world data, and to further analyze incidence and trends in Costa Rica (Paper I).

- To develop high-quality methods for the assessment of past exposures to pesticides in individual agricultural workers, feasible in a developing country (Paper II).

- To integrate exposure data from direct and external sources in a formal model for the assessment of occupational pesticide exposure (Paper III).

- To assess qualitative and exposure-response relations between parental occupational exposure to pesticides and risk of leukemia in the offspring (Paper IV).

4 MATERIALS AND METHODS

4.1 Paper I

The study population comprises the entire population of children under 15 years of age during the period 1981-1996 in Costa Rica.

The individual data of all newly diagnosed cases of leukemia (ICD-0) in the study population during the period January 01, 1981 through December 31, 1996 were extracted from the RNT records. We constructed a case database that included gender, year of diagnosis, age at diagnosis, histology, and region of residency in Costa Rica at the time of diagnosis.

Annual estimates of the size of the study population, stratified by gender, age, year and region were obtained for the year 1984 from the Costa Rican census data for that year, and from annual sample survey data and annual numbers of births and deaths, as compiled by the Central American Population Program of the Universidad de Costa Rica, for the remaining years.

Incidence rates were calculated per million person-years and analyzed for histology, gender, time period at diagnosis, age at diagnosis and region. The 22 geographical regions, defined by the Ministry of Health for public health purposes, were collapsed into seven larger regions similar in economic activity, climate, altitude and population density. Regional incidence rates were age standardized using the age structure of the Costa Rican national population as the standard. A Poisson regression model was fitted for the incidence of ALL, with region, calendar period, gender and age as predictors. Log of person-years was taken as offset in the model.

An international comparison of rates was also performed, using the data from *International Incidence of Childhood Cancer*, published by IARC (Parkin et al. 1998).

4.2 Paper II

We describe an icon-calendar interview form (ICF) for a case-control study of childhood leukemia and parental exposure to pesticides. The ICF is a pictorial format adapted for the Costa Rican population, using icons of special life events, crops, geographical regions and application techniques such as drawings to enhance recall in the interviewees. It includes calendar sheets, icon stickers, icons shown to the interviewees, markers for durations of exposure patterns, and checklists of specific pesticides. Using the ICF, we collected data on parental agriculture related use of pesticides, agricultural job tasks, frequency of exposure (number of applications per month and hours/day), major determinants of pesticide exposure (task-technology, personal protective equipment, field reentry, storing of pesticides, personal hygiene). These data were collected on a month-

to-month basis from two years before birth until diagnosis of index child (leukemia cases) or until either interview date or age 15 (controls).

Checklists of pesticides were constructed for crops and time periods to facilitate recall. These were shown to the interviewees after they had spontaneously reported the use of pesticides they remembered. The pesticides in the checklists were selected from the prioritization system (Valcke et al. 2005) described in Section 2.3.1, based on toxicity and frequency of use. The checklists contain both trade names and active ingredients, grouped by biocidal action (insecticide, nematocide, herbicide, fungicide, other).

Evaluation notes were recorded by the interviewers for each interview to assess the performance of the form. This included duration of the interview, overall degree of difficulty in applying the method (easy-moderate-difficult), degree of familiarity of the subject with the icons, and his or her ability to recall life events and occupational information.

4.3 Paper III

Two models for retrospective exposure assessment were constructed for interviewees who worked in agriculture or were involved in pesticide application on livestock at any time during the exposure assessment period of the case-control study: a quantitative model and a qualitative model.

The quantitative model was constructed for individual parental exposure to 25 pesticides. The selection of the pesticides was based on toxicity-based prioritization system (Valcke et al. 2005; Section 2.3.1). Table 3 presents a list of the prioritized pesticides used in the model.

Table 3. List of pesticides included in the study by biocide category, active ingredient, chemical group and carcinogenicity status. IARC: International Agency for Research on Cancer. EPA: Environmental Protection Agency.

Biocide category/ active ingredient	Chemical group	Carcinogenicity status[1]
Herbicides		
2,4-D	Phenoxyacetic acid	2B (IARC); D (EPA)
Diuron	Chlorinated urea	NC (IARC; D (EPA)
Fluazifop	Phenoxy, trifluorimethil pyridine	NC (IARC; H (EPA)
Glyphosate	Phosphoric acid	NC (IARC; E (EPA)
Picloram	Pyridine	3 (IARC); D (EPA)
Paraquat	Bipyridil	NC (IARC; E (EPA)
Terbuthylazine	Triazine	NC (IARC; H (EPA)
Insecticides		
Carbofuran	Carbamate	NC (IARC; E (EPA)
Deltamethrin	Pyrethroid	3 (IARC); D (EPA)
Dichlorvos	Organophosphate	2B (IARC); E (EPA)
Fenamiphos	Organophosphate	NC (IARC; D (EPA)
Malathion	Organophosphate	3 (IARC); D (EPA)
Methamidophos	Organophosphate	NC (IARC; D (EPA)
Methomyl	Carbamate	NC (IARC; D (EPA)
Oxamyl	Carbamate	NC (IARC; E (EPA)
Phoxim	Organophosphate	NC (IARC; D (EPA)
Terbufos	Organophosphate	NC (IARC; D (EPA)
Fungicides		
Benomyl	Benzimidazole	NC (IARC; C (EPA)
Captafol	Chlorinated phthalides	2A (IARC); C (EPA)
Chlorothalonil	Benzonitril	2B (IARC); B(EPA)
Cyproconazole	Conazole	NC (IARC; D (EPA)
Lead arsenate	Arsenical	1 (IARC); D (EPA)
Mancozeb	Dithiocarbamate	NC (IARC; D (EPA)
Quintozene	Organochlorine	NC (IARC; D (EPA)
Triadimefon	Conazole	NC (IARC; C (EPA)

1- IARC classification: Group 1: carcinogenic; Group 2A: probably carcinogenic; Group 2B: possibly carcinogenic; Group 3: not classifiable; Group 4: probably not carcinogenic to humans. EPA classification: A: known carcinogenic; B: probably carcinogenic; C: possiby carcinogenic; D: not classifiable; E: not carcinogenic.

The model combined ICF interview data (Paper II) and external data on pesticide use. From the ICF data, we collected monthly data on frequency of application, agricultural jobs, task and technology, personal protection, time of reentry to sprayed area and storing of pesticides. These primary data were converted into individual hazard scores C_{ti} over each determinant category d during time window t for subject i.

Relative weights w_d and individual time-specific hazard values H_{dti} for each determinant category d were determined on a judgmental basis, i.e., the w's and H's were not data-driven. C_{ti} took the form:

$$C_{ti} = \sum_{d=1}^{5} w_d H_{dti} \qquad (\Sigma\, w_d = 1)$$

An external database provided information on the application rates (used as proxy for intensity of potential exposure) for each pesticide, obtained from a historical pesticide use database for Costa Rica, constructed at the Central American Institute for Studies on Toxic Substances of Universidad Nacional (IRET-UNA), mentioned in section 2.3.3.

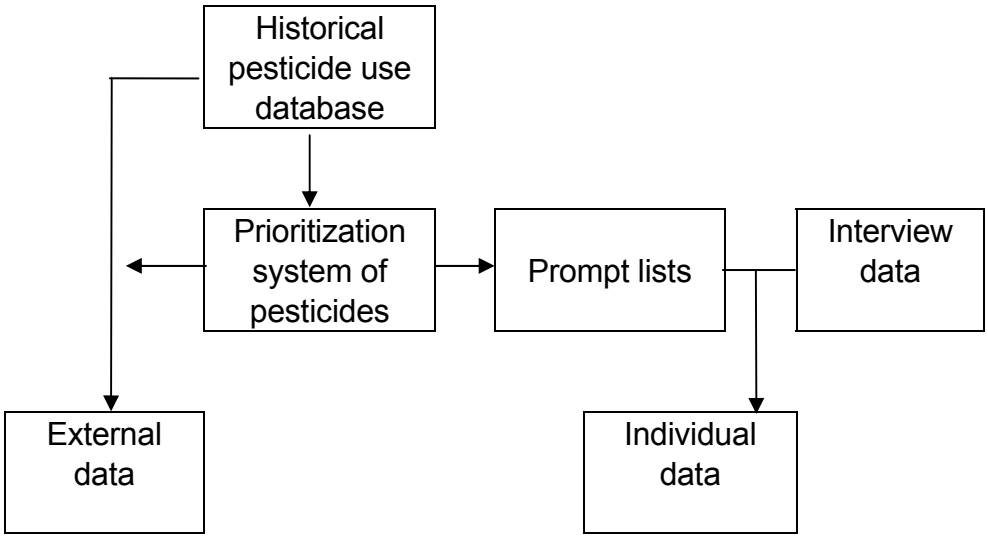


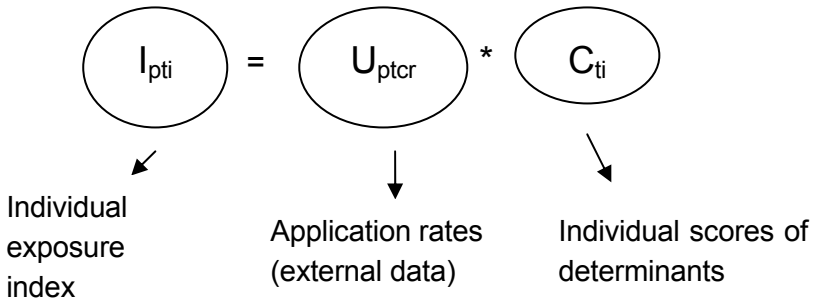
Figure 2. Data sources

The external data were scrutinized by two experts to convert all units into liters/hectare of active ingredient. This quantity was denoted by U_{pycr}, where p = pesticide (1,2,…,25; Table 1); y = calendar year (1982 … 2002); c = crop (1,2,…,14[1]); and r = geographical region (1, 2,…14). We converted the calendar time y into individual time that had birth of the child as the "zero time" reference. The time-converted metric was denoted as $U_{ptcr,}$ where t took on five values: year

[1] Rice, banana, coffee, sugar cane, onion, melon, orange, ornamental and ferns, potato, pineapple, tomate, macadamia, beans and pastures.

before conception; 1st trimester; 2nd trimester; 3rd trimester; and first year of life of the child.

The individual exposure index I_{pti} was calculated as:



The qualitative model assigned simple binary indicators of occupational exposures in agriculture. These were derived from the interview data and, whenever necessary, augmented by external data. The model covered 14 chemical groups of pesticides for all time windows, where any exposure to one of the compounds of the chemical group was sufficient to trigger "exposure". The chemical groups included also the generic group "other pesticides".

4.4 Paper IV

4.4.1 Study population and study design

The study population for the case-control study comprises the entire population of children of Costa Rica under 15 years of age, at any time during the period 1995-2000.

4.4.2 Data collection

4.4.2.1 Selection of cases and controls

All cases of childhood leukemia (ages 0-14 at diagnosis; N= 334) diagnosed in Costa Rica during the period January 01,1995 through December 31, 2000 were identified at the RNT and the Children's Hospital of Costa Rica (HNN) (ICD-0). Population controls (N=579), frequency matched to the cases by birth year, were drawn from the National Birth Registry, using computerized random selection. Diagnoses of cases were extracted directly from the Cancer Registry data and confirmed with HNN files. For the purposes of this study, the acute lymphocytic leukemias (ICD-O-2 9821-9827 as diagnosed by the RNT and the HNN) were treated as a case subcategory. The effective number was 300 cases and 579 controls.

Since in Costa Rica landmarks are used to indicate addresses instead of street names and house numbers, a standard procedure to locating families while maintaining randomness, was developed. Addresses and occasionally telephone numbers of cases were obtained from the National Children's Hospital files. This information was adequate to trace the family in the majority of the cases. When

the information was inadequate, we used databases of the local Social Security clinics. For controls, we checked in the electoral databases the locality where the mother had voted in the last presidential elections, and visited, with this information, the local Social Security clinics which usually were able to provide exact addresses. If we did not find the family but obtained information on a new address, we attempted to locate the family at the new address. If this failed, the control child was replaced with a child within the same age range (± 1 year) living in the same neighborhood. If the address was too vague to be traceable and the family was not known at the health center of the neighborhood, a control child of the same age (± 1 year) was randomly selected from the archives of the health center. Including the refusals (19 cases), the total losses from the original case series were 34 in number (10.2%), with obviously no replacement. For the controls, the unlocalized (215) and refusals (55) were all replaced, the replacement rate thus being 46.6%. There were no cancers in the controls.

Face-to-face interviews of parents were conducted during 2001-2003. A computerized data management system was established for tracing the families, because the interviews required information on the father, the mother and the index child, and sometimes parents did not live together and/or in the same location, or the father could not be identified. In the final case series, 267 (89.0% included data for both parents; in the final control series, this number was 492 (85.0%).

4.4.2.2 Agricultural exposure and its determinants

Exposure data were collected using two interview forms: a conventional and an icon-calendar form (Paper II). The time period for exposure assessment was taken as the period from 12 months before conception until diagnosis of the cancer for cases until either the interview date or age 15 of the controls, whichever occurred first.

A *conventional interview form* was offered to both parents. It had three versions: for father, mother and child (the latter completed by either parent). The interview included family residence location; education of father and mother; smoking and substance abuse of father and mother; diet of mother; medical history of mother including vaccinations; mother´s X-rays and medications; pregnancy history; birth parameters; occupational, environmental and home pesticide exposures of both parents; occupational histories of parents; and exposures to non-pesticide toxic agents of fathers and mothers.

Parents who were active in agriculture and/or livestock breeding during the assessment period completed an additional interview, which utilized an *icon-calendar form* (ICF) (Section 4.2). Using the ICF, we collected data on parental use of pesticides, agricultural job tasks, frequency of exposure (number of applications per month and hours/day), major determinants of pesticide exposure (task-technology, personal protective equipment, field reentry, storing of pesticides, personal hygiene) for the etiologically relevant time period on a month-to-month basis.

4.4.3 Epidemiological data analysis

Unconditional crude (bivariate) and adjusted logistic regression models were used to estimate odds ratios (OR) and their 95% confidence intervals (CI), separately for the five etiologically pertinent time windows (Section 5.3). For pre-term births, an adjustment was applied for the estimation of conception date. Qualitative (yes/no), semi-quantitative (unexposed / low exposure / high exposure), and quantitative estimates I_{pti} of specific pesticide exposure levels were treated as independent variables. Analyses were performed also for groups of pesticides. A correlation matrix for 25 prioritized pesticides, selected risk factors and chemical groups of pesticides, computed in the controls, was used to select the potential counfounders in the logistic models. Overall low correlations resulted in the inclusion only of the urban/rural residence in all logistic models for the purpose of controlling for unmeasured urban or rural risk factors. Mother's X-ray exposure during pregnancy was included in the phtalide model because of its moderate correlation with exposure to these pesticides. The unexposed were the reference group in all analyses, except for the analysis between high and low exposure groups, where the low exposure level was the reference level.

5 RESULTS

5.1 Childhood leukemia in Costa Rica: international comparison (Paper I)

During 1981-1996, 918 cases of leukemia in children under 15 years (510 boys, 408 girls) were reported to the RNT of Costa Rica. They represented 41% of all reported cancers in children, with an overall age-standardized incidence rate of 55 per million person-years (59 in boys and 49 in girls). Acute lymphocytic leukemia (ALL) rate was 43 per million person-years and represented 79% of the total leukemias. Acute nonlymphocytic leukemia (ANLL) rate was 9 per million and represented 16% of total leukemias. ALL rate for boys aged 1-4 years was 76 per million.

There were downward time trends in incidence of total leukemias, ALL and ANLL and "not otherwise specified" (NOS) during the period. The identified risk factors for ALL were male gender, ages 1-4 and 5-9 years, and urban residence.

The observed rates were among the highest in the world, especially for ALL among boys 1-4 years old. Costa Rican rates for lymphoid leukemias in boys (which are 99% of lymphocytic leukemias) was 52 per million person-years for years 1984-1992 and exceeded those reported for Europe (22-47 per million), most North America (18-50 per million), Oceania (30-48 per million), Africa (13 per million) and Asia (3-51 per million). Costa Rican rates were comparable only with Hong Kong (51 per million) and Hispanics in Los Angeles, US (50 per million). The comparisons were based on the data of the International Agency for Research on Cancer (IARC) (Parkin et al. 1998).

5.2 Performance of the ICF (Paper II)

The performance of the first 100 completed ICF interview forms was reported. These corresponded to 42% for cases and 58% for controls. Eighty-five percent of the respondents were men; 50% were 40 year of age and younger; and 82% had attended primary school only. Duration of interviews ranged from 10 to 90 minutes, depending on the length of the assessment period of exposure and complexity of work histories. The overall performance in the application of ICF, recorded by the interviewers, was considered "easy" in 58% of the interviews, "moderately easy" in 33% and "difficult" in 8%.

General questions regarding time periods of working in agriculture and on exposures to pesticides by biocide categories were included in the conventional interview form and in ICF. These two sets of data were consistent in 92% of the subjects.

Recall was judged acceptable in 90% of the interviews. The quality of the ICF was evaluated with the following criteria: successful identification of special events (84%); satisfactory recall of data on crops and jobs tasks (84%), pesticide

use (names, frequencies, application techniques) (90%); pesticide use in reference to changes to jobs and tasks (62%); changes in pesticide exposure patterns with reference to a special event (63%); and reported frequency of changes in pesticide use during the assessment period (63%). The usefulness of pesticide checklists in prompting further pesticides was satisfactory for 78% of the interviews.

Ninety percent of those who were asked about whether they liked the instrument responded positively. Reasons included "interesting method", "a way to squeezing my brain for recall" and "interactive method".

5.3 Exposure intensities of parents exposed to pesticides (Paper III)

With the exposure assessment model, we calculated the numbers of exposed fathers and mothers for each prioritized pesticide and its exposure intensities. These were calculated for 5 time windows (adapted for each individual calendar time): year before conception, 1st, 2nd and 3rd trimesters, and the first year of life of the child.

The numbers of exposed fathers were overwhelmingly higher than of exposed mothers. Median exposure intensities among exposed were, for some pesticides, higher for fathers and for others, higher in mothers.

Exposure intensities during active exposure and nonactive exposure time combined were compared. High correlations between the two indicators of exposure intensity were found for deltamethrin, aldrin, fluazifop and carbofuran. Correlations <0.4 were found for 2,4 D, benomyl, paraquat, mancozeb, terbufos, triadimefon, lead arsenate, dichlorvos, terbuthylaine and quintozene.

A sensitivity analysis of the exposure model was done by changing the weights w maximally for each determinant within realistic limits. The hazard values H were assumed to be reasonably valid and therefore were not subjected to sensitivity analysis.

For the sensitivity analysis a random number generator produced 10,000 sets of H_{di} from uniform distributions of each d. Original and changed weights were then applied to each of the 10,000 sets. Two C scores were thus calculated for each set and intercorrelated (Section 4.3). The sensitivity analysis between the two C exposure scores resulted in a correlation coefficient 0.91 between the two sets, suggesting satisfactory robustness of the weighting.

5.4 Risk of childhood leukemia associated with parental occupational exposure to pesticides (Paper IV)

A total of 879 families were included in the analysis (300 cases and 579 controls), with data from 876 mothers and 762 fathers.

Cases and controls were similar in maternal age at conception, proportion of parents exposed to pesticides, maternal smoking and alcohol consumption during pregnancy, and maternal education. Cases were more often boys, and their families represented slightly lower socioeconomic status than controls. Acute lymphocytic leukemia (ALL) represented 83.7%, acute non-lymphocytic leukemia 13.7% and "others" and "not otherwise specified" leukemias 2.7%. The proportion of parents who worked in agriculture was similar for cases and controls.

Odds ratios for all pesticides by biocide category (insecticides, herbicides and fungicides) during the 5 time windows, separately for mothers and fathers were calculated. Significant excesses for mothers were found for all pesticides combined during the year before conception and 1st and 2nd trimesters (OR 2.4, 95% CI 1.0-5.9; 22, 2.8-171.5 and 4.5, 1.4-14.7), and anytime (2.2, 1.0-4.8); insecticides year before conception (4.6, 1.2-17.8), 1st trimester (OR undefined), 3rd trimester (3.4, 1.0-11.8) and anytime (3.0, 1.0-8.4); herbicides during 1st and 2nd trimesters (13.8, 1.7-112.9 and 4.6, 1.2-17.9), and fungicides anytime (1.9, 1.1-3.0). Fathers' exposures showed significant excess risk for all pesticides during 2nd trimester (1.5, 1.0-2.3); insecticides during 3rd trimester (2.2, 1.2-4.1), herbicides during 2nd trimester (1.6, 1.0-2.5); and fungicides before conception (1.6, 1.0-2.6), and anytime (1.9, 1.1-3.0). Table 4 shows the ORs for exposures at anytime of fathers and mothers separately, by use of all pesticides and by biocidal group of pesticides.

Table 4. Odds ratios (OR) and 95% confidence intervals (95% CI) for total childhood leukemia and exposure at anytime of fathers and mothers, by use of pesticide by classes of biocidal action adjusted for residence (urban-rural). NEC: Number of exposed cases.

Biocide group	Fathers			Mothers		
	NEC	OR	95% CI	NEC	OR	95% CI
All pesticides	66	1.4	0.9-2.0	13	2.2	1.0-4.8
Insecticides	44	1.4	0.9-2.1	9	3.0	1.0-8.4
Herbicides	60	1.4	0.9-2.0	11	1.4	0.9-2.0
Fungicides	36	1.9	1.1-3.0	6	1.9	1.1-3.0

Risks of exposure to pesticides by chemical group were calculated for mothers and fathers (Table 5). Significant associations were found for mothers for organophosphates for 1st trimester for total leukemias (3.5, 1.0-12.2) and 1st and 3rd trimester for acute lymphocytic leukemia (ALL) (3.7, 1.0-13.1 and 3.3, 1.1-9.6); and for fathers for total leukemia for organophosphates during the year before conception and 1st trimester (1.5, 1.0-2.2 and 1.6, 1.0-2.6), and benzimidazoles during 1st, 2nd and 3rd trimesters of pregnancy (2.2, 1.0-4.4; 2.2, 1.0-5.0 and 2.2, 1.0-5.2).

Table 5. Odds ratios (OR) and 95% confidence intervals (95% CI) for total leukemia for fathers' and mothers' reported use of pesticide by chemical group, adjusted for residence (urban-rural). NEC: Number of exposed cases. Only chemical groups with at least one significant result in any time window included.

	FATHERS			MOTHERS		
	NEC	**OR**	**95% CI**	**NEC**	**OR**	**95% CI**
Year before conception						
Organophosphates	48	1.5	1.0 -2.0	9	2.3	0.9 -6.0
Benzimidazoles	19	1.8	0.9 -3.0	1	-	-
Others[1]	56	1.1	0.8 -1.6	11	2.8	1.1 -7.0
1st trimester						
Organophosphates	33	1.6	1.0 -3.0	7	3.5	1.0 -12.0
Benzimidazoles	14	2.1	1.0 -4.0	1	-	-
Others[1]	38	1.1	0.7 .1.7	9	3.7	1.2 -11.0
2nd trimester						
Organophosphates	31	1.4	0.8 -2.0	5	2.5	0.7 -10.0
Benzimidazoles	13	2.2	1.0 -5.0	1	-	-
Others[1]	37	1.1	0.7 -1.8	8	3.3	1.1 -10.0
3rd trimester						
Organophosphates	30	1.5	0.9 -3.0	8	2.7	0.9 -8.0
Benzimidazoles	12	2.2	1.0 -5.0	1	-	-
Others[1]	36	1.1	0.7 -1.8	9	3.7	1.2 -11.0
1st year of life						
Organophosphates	43	1.3	0.9 -2.0	7	1.8	0.6 -5.0
Benzimidazoles	16	1.7	0.9 -4.0	1	-	-
Others[1]	56	1.1	0.8 -1.7	9	2.1	0.8 -5.0

1- Mainly paraquat, chlorothalonil and glyphosate

There was a suggestion of an exposure-response gradient between low and high exposure level, in risk for fathers for picloram for total leukemias before conception and during the first year of life. Exposure-response gradients were also found for fathers exposed to benomyl and total leukemia and ALL during the first year of life of the child, and paraquat exposures and total leukemia during the year before conception.

Age at diagnosis tended to be positively associated with fathers' exposures and inversely with mothers' (Table 6).

Table 6. Odds ratios (OR) and 95% confidence intervals (95% CI) for total leukemia and reported use of any pesticides by time window and age at diagnosis, adjusted for residence (urban-rural). NEC: Number of exposed cases.

Time window for exposure	Age at diagnosis, years					
	1-5			6-15		
Fathers						
Total pesticides	**NEC**	**OR**	**95% CI**	**NEC**	**OR**	**95% CI**
1 year before conception	26	0.8	0.5- 1.3	37	1.8	1.1- 2.9
1st trimester of pregnancy	20	0.9	0.5- 1.6	24	1.4	0.8- 2.4
2nd trimester of pregnancy	21	1.1	0.6- 1.8	24	1.6	0.9- 2.6
3rd trimester of pregnancy	18	0.9	0.5- 1.7	18	1.2	0.7- 2.1
1st year of life	26	0.9	0.5- 1.4	33	1.6	1.0- 2.5
Mothers						
Total pesticides						
1 year before conception	7	3.0	1.1- 8.3	4	2.0	0.6 -6.6
1st trimester of pregnancy	7	27.6	3.4-226.1	4	18.0	2.0 -162.2
2nd trimester of pregnancy	6	5.8	1.6- 21.1	3	3.3	0.7-15.2
3rd trimester of pregnancy	6	2.9	1.0- 8.5	3	1.6	0.4 -6.4
1st year of life	6	2.3	0.8- 6.5	4	1.8	0.5- 5.8

6 GENERAL DISCUSSION

Our study provides epidemiological data of childhood leukemia in the Costa Rican population, using both descriptive and etiological approaches. We started from national statistics data on childhood leukemia; we developed pesticide-specific exposure assessment procedures and used it for the epidemiological analysis in a population-based case-control study. These data contribute to an improved understanding of some risk factors associated with leukemias in children, which may also be extrapolated to other populations with similar risk factor profiles. It also offers innovative methods for exposure assessment, using the available information in the country.

Costa Rica combines features of a developing and developed country. It offers national registries and data sources of reasonably high quality for epidemiological research, such as the National Civil Registry (births and deaths), census and intercensus sample survey data on the population and households, electoral databases, pesticide import and use databases (Wesseling & Bravo 2002), a database on carcinogenic occupational exposures (Partanen et al. 2003, Chaves et al. 2005), a national cancer registry, social security databases, hospital records, and a congenital malformation registry.

6.1 Childhood leukemia incidence (Paper I)

Childhood leukemia incidence was found to be similar to those reported in other countries in its distribution by age, gender and histology type, but Costa Rican rates were high. Male gender, age 1-4 years, and urban residence were identified as risk factors.

The quality of the RNT meets international standards, which is reflected in the Costa Rican data having been included in the IARC international cancer incidence statistics for the past 20 years (Muir et al. 1987, Parkin et al. 1992, Parkin et al. 1997, Parkin et al. 2003).

An observed downward time trend in incidence of ALL during 1981-1990 seems not to result from diagnostic misclassification, as the decreasing trend is seen also when ALL and NOS leukemias are combined. Also, these data were scrutinized and checked for inconsistencies. High standards of medical specialists at the HNN are long established, and diagnoses were based on bone marrow smears for 98% of the cases in 1996. We do not have an explanation to the decreasing trend in Costa Rica, given increasing trends or no change in some other countries as Great Britain, US and Nordic countries (Smith et al. 1999, Kroll et al. 2006, Lyngsie et al. 2004).

Possible causes for male excess in childhood leukemia are unknown. It has been suggested that boys are more likely to have T-cell ALL than girls, and also that the risk is related with boys having higher birth weight than girls and thus

higher number of cells at risk in the tissue from which the leukemia arises (Doll & List 1989, Imbach et al. 1995).

6.2 Evaluation of ICF calendar for assessing past parental occupational pesticide exposure (Paper II)

In general, validation of retrospective exposure data is difficult. In particular, the validity of self-reported exposures to specific agents and trade names of chemicals appears low (Teschke et al. 2002).

Judging from the small amount of data available for validity assessment, our ICF format seems to perform satisfactorily. Thus, the ICF and our conventional interview agreed at 92% consistency on time periods worked in agriculture and on exposures to herbicides, insecticides and fungicides. Easily recalled events facilitated timing of recall. Job data being satisfactory, data on pesticides (though clearly improved by pesticide checklists) remained more deficient and were augmented with auxiliary external data in the final assessment of individual exposures.

The quality of the ICF data was evaluated using various parameters (Section 5.2). The ICF performed well for recall, special events, crops and tasks and timing, type and application of pesticides; and less well for reporting changes in pesticides over time.

Life-event calendars and icon-based interviews have been applied in agriculture in other studies, and the feasibility of the method in a developing country setting appears satisfactory.

6.3 Evaluation of the exposure assessment model (Paper III)

The validity of the exposure model cannot be directly assessed in the lack of any meaningful empirical criterion data. The validity issue will therefore be approached by a critical scrutiny of the major components of the model and the form of the model.

First, the application rates were specific to combinations of crop, pesticide, calendar year and region. These data were further re-evaluated by experts for the purposes of the study. The external component of the exposure model thus is to a fair degree contextual, reflecting a variety of concrete exposure situations.

An important validity consideration pertains to the question of to what extent the application rate, expressed as volume of active ingredient per surface area, can be interpreted as a valid substitute for the intensity of potential individual exposure. It seems obvious that increasing rate of sprayed pesticide volume would increase the probability and intensity of potential individual exposure. However, application technology and other determinants modify the general exposure potential. The important modifying factors or individual determinants of exposure, assessed by the interviews, were therefore expressly built into the

model, thus individualizing the exposure potential estimated from external data on application rates.

The hazard values (H) were based on an inter-expert consensus after several group discussions on different candidate sets of values. The experts included persons with field experience in Costa Rica.

Different model forms were considered, and an additive form was chosen since it considers all hazards and provides, as it was felt, an appropriate weighting to high-level hazards. A sensitivity analysis of the model demonstrated robustness of the model.

6.4 Validity of the case-control study (Paper IV)

A number of associations were found for particular pesticides and groups of pesticides for mothers' and fathers' exposures during the different time windows. The validity of these findings is discussed below.

Both cases and controls were selected from the general population. Cases and controls were in no manner matched on parents' economic activity such as agriculture. The procedure for selecting and locating the controls therefore seems to ensure representation of the general infant/child population from which the cases were identified.

Replacement of controls was done by age group and place of residence of the original control child. Thirty-four cases were not included in the study: they refused to participate or were not localized. Comparing data on urbanity/rurality of residence and years of diagnosis between the excluded and included cases does not suggest bias from case exclusion. We do not believe that the control replacement procedure (270 controls replaced) introduced a bias, since the replacement was done in a generally accepted manner.

Our model for exposure assessment involved a procedure for enhancing recall. Differential misclassification of exposures between parents of cases and parents of controls, due to interview-based assessment, is possible. An increased sensitivity (probability of finding true positives) of the exposure assessment is possible, with an ensuing decrease in specificity. These effects are probably non-differential between cases and controls and would tend to bias the ORs toward unity. An expert check of reported pesticides eliminated a number of false positives, which counteracted an unknown amount of loss in specificity.

We calculated correlation coefficients in the controls between exposure of a number of potential confounders, the exposures to specific pesticides, and groups of pesticides. This procedure allowed us to prioritize the factors to be controlled in the logistic models, discarding variables with low correlations with the pesticide variables.

Mothers' and fathers' exposures are to a certain degree interdependent because of exposures to same pesticides, and possibly due to similarity in the response patterns in the interview. The Spearman rank correlation coefficient between mothers and fathers for ORs for insecticides, herbicides and fungicides in the five time windows was however moderately low (0.32).

It remains uncertain whether case mothers and fathers would recall their job details (which were the basis for the assessment of their exposures) better than those of the controls. This leaves room for a possible positive bias. Exposure assessment was blinded as of the case-control status of the children, being a computerized modelling procedure, thus not inducing a differential assessor bias.

We assessed exposures for fathers and mothers separately and integrated interview information with external data on time-, locality- and crop-specific pesticide application practices (Papers II and III). The excess risks in our study were strongest for mothers and were found for the different types of biocides and several chemical groups. These effects were not evident for specific pesticides, except for paraquat, since the numbers of exposed mothers were small. Besides, mothers' exposures were more relevant for leukemia diagnosis during first 5 years of life, while fathers' exposures appeared to be associated with later diagnosis. We do not have data for explaining this phenomenon, which does not seem to be caused by biases, as explained above.

6.5 Consolidated evidence

The observed rates of childhood leukemia in Costa Rica are among the highest in the world, particularly for ALL among boys aged 1-4 years, and they reflect particular risk factors in the population, of which we chose to study pesticides, as their use is frequent and intensive in Costa Rica. There are consistencies and also novel findings in our study, considering previous world evidence.

In general the role of parental occupational exposures in the development of childhood leukemia still remains relatively unclear (Buffler et al. 2005). Pediatric leukemias have multifactorial etiologies involving the interactions between environmental aspects as well as human genetics (Greaves 2002). Previous studies in other populations have reported associations of childhood leukemia with environmental exposure to pesticides. Children of farmers and farm workers are exposed to agricultural chemicals *in utero* through transplacental transmission during gestation, and also during the postnatal period by diverse pathways: they live and/or work on farms with their parents and come into contact with agricultural chemicals through direct contact with plants, soil and stored pesticides; also through their parents take-home exposures; and direct physical contact with their parents through breath, working clothes and skin (Curl et al. 2002). Other environmental exposures to pesticides are also possible, such as indoor exposures or contact with pets treated with pesticides.

Epidemiological studies of pesticides and childhood leukemia have been reviewed by Zahm & Ward 1998, Daniels et al. 1997 and Buffler et al. 2005,

among others. Thirty previous epidemiological studies on pesticide and childhood cancer have been conducted. These include 25 case-control studies, 4 cohort studies, and one ecological study. The results have been summarized in Appendix 1. Three case studies have been added.

Two epidemiological studies (Shu et al. 1988, China; Meinert et al. 2000, Germany) found moderately high excesses associated with maternal unspecified farming jobs during pregnancy. For paternal farming jobs or associated pesticide exposures, excesses were reported in the U.S. (Buckley et al. 1989), U.K. (Gardner et al. 1990), Italy (Magnani et al. 1990), Canada (Infante-Rivard & Sinnett 1996), and Germany (Meinert et al. 2000).

For unspecified occupational or nonoccupational pesticide exposure, positive findings were found by Petridou et al. (2000) for mothers in Greece and Abadi-Korek et al. (2006) for unspecified parental occupational exposure.

Nonpositive findings for parental occupational titles or unspecified pesticide exposure were reported by Hemminki et al. (1981) in Finland, Gold et al. (1982) in the U.S., Laval & Tuyns (1988) in France, Van Steensel-Mol et al. (1985) in the Netherlands, Infante-Rivard et al. (1991) in Spain, Meinert et al. (1996) in Germany, Roman et al. (1993) in the U.K., Kristensen et al. (1996) in Norway, Feychting et al. (2000) in Sweden, Rodvall et al. (2003) in Sweden, and Flower et al. (2004) in the U.S.

Significant exposure-response for frequency of household pesticide exposure of the child and mother in the U.S. was reported by Buckley et al. (1989), and an excess risk was found for familial garden (Meinert et al. 1996) or farm (Meinert et al. 2000) exposure in Germany.

Nonpositive findings were reported for parental gardening with pesticides in the U.S. (Schwartzbaum et al. 1991) and Germany (Meinert et al. 2000); child's environmental pesticide exposure in Canada (Deschamps et al. 1993); yard pesticide treatment in the U.S. (Leiss & Savitz 1995); and house extermination in the U.S. (Leiss & Savitz 1995).

Perhaps the most consistent excesses were reported for insecticides as a group. Excesses were reported for exposure during the last trimester in the U.S. (Leiss & Savitz 1995); during pregnancy and childhood in Canada (Infante-Rivard et al. 1999); for household insecticides in Germany, with an exposure-response gradient (Meinert et al. 2000), and in France (Menegaux et al. 2006); and for household and other pest control insecticides in the U.S. at a number of time windows including pregnancy (Ma et al. 2002). Pediculosis treatment with insecticidal shampoo was associated with an excess risk in France (Menegaux et al. 2006). Nonpositive results for insecticides were reported for Spain (Infante-Rivard et al. 1991). Our study found a threefold excess associated with mothers´ occupational exposure to insecticides.

Excesses were found for garden herbicides by Infante-Rivard et al. (1999) in Canada during pregnancy and childhood. Canadian (Infante-Rivard & Sinnett 1999) and U.S. (Ma et al. 2002) findings for herbicide exposure before and during pregnancy were excesses of borderline significance, which is in accordance with our results for occupational herbicide exposure.

Results for fungicides as a group are consistent but based on two studies only, in Canada (Infante-Rivard & Sinnett 1999) and France (Menegaux et al. 2006). Infante-Rivard et al (1999) reported a fivefold excess associated with preconceptional exposure to fungicides in Canada. Our study found a twofold significant excess associated with mothers´ and fathers´ occupational exposure to fungicides.

A slight borderline significant excess associated with organophosphate pesticides was found in the U.S. (Reynolds et al. 2005), but not in another ecological U.S. study (Reynolds et al. 2002). The latter study reported nonpositive results also for carbamates, dithiocarbamates, and organochlorine pesticides. We found suggestions for excess risk associated with occupational exposure to organophosphate pesticides for both mothers and fathers.

Pediculosis treatment with pyrethroids was associated with an excess risk in France (Menegaux et al. 2006).

Benzimidazoles, for which we found excesses for fathers, were not studied in other studies.

For particular pesticides, Scheele et al (1992), measured bone marrow levels of DDT, DDE, hexachlorobenzene, hexachlorocyclohexane and dieldrin in bone marrow of children in a small case-control study in Germany, with nonpositive results throughout. In the French study on pediculosis treatment, the excess for lindane was statistically unstable, and for malathion the evidence was nonpositive.

Reynolds et al (2002, 2005) reported on two studies in the U.S. Excesses were reported for propargite, metham sodium and dicofol, but only in one of the studies each. No clear excesses were seen for methyl bromide, trifuralin, simazine or chlorothalonil.

The case studies reported exposure in cases from home treatment with chlordane (Infante et al. 1978) and dichlorvos and propoxur (Reeves et al. 1981; 1982). All were U.S. studies.

In our study we found excesses particularly for picloram, benomyl, foxim, paraquat, mancozeb and malathion.

7 CONCLUSIONS AND RECOMMENDATIONS

Considering our data in conjunction with worldwide data, we believe that there are reasons for supporting associations between parental and early childhood pesticide exposures and the risk for childhood leukemia. The most likely candidates for etiological pesticidal compounds appear to be -still with considerable uncertainty- benomyl, picloram, paraquat, foxim, mancozeb, malathion, dichlorvos and metam sodium. One of the uncertainties derives from the fact that the compounds we studied were not studied in previous epidemiological studies, with the exceptions of malathion (Menegaux et al. 2006). Dichlorvos was previously reported only in case reports (Reeves et al. 1981, Reeves et al. 1982). However, there may be reason for re-evaluating the carcinogenicity of some of these pesticides by evaluating agencies.

We believe that epidemiological research on pesticide exposure and cancer, including parental exposure and childhood cancer, needs to be further conducted among populations with possibilities for high-quality exposure assessment and exposure contrast, preferably in agricultural settings without excessive changes over time of the pesticide use pattern, which, on the other hand, have been difficult to identify.

In Costa Rica, further studies need to be conducted to evaluate the association of the high rates of childhood leukemia with domestic pesticide exposure, as well as exposures to other chemical (such as disinfectant compounds) or physical (such as electromagnetic fields) agents.

There is also a need to study biomarkers for assessing biological processes in leukemogenesis, susceptibility and exposure in molecular epidemiology in Costa Rica.

We recommend prevention on pesticide hazards especially in the population stratum reproductively active and also among children. The C139 Convention of the International Labour Organisation (ILO), concerning “Prevention and Control of Occupational Hazards caused by Carcinogenic Substances and Agents”, was proclaimed in 1974, taking into account the relevant work of WHO and IARC regarding cancer research. The Convention has been ratified by a number of countries, but not Costa Rica. It establishes the following issues:

- Periodic listing of carcinogenic substances that would be prohibited or made subject to authorization and control.
- Replacement of carcinogenic substances.
- Exposure minimization.
- Appropriate system of records.
- Informing workers of dangers and protection.
- Appropriate medical examinations or biological or other tests for investigations.
- Codes of industrial/agricultural conduct.

All these measures are relevant in the prevention of childhood cancer through parental occupational exposures, such as carcinogenic pesticides.

Human data allows imperfect evaluation of carcinogenicity of pesticides, generally due to exposures to multiple agents. IARC has classified ethylene oxide, chromium (VI) containing wood preservatives and arsenical pesticides in Group 1 (Carcinogenic to humans). Group 2A (Probably carcinogenic to humans) includes captafol, ethyl dibromide and nonarsenical insecticides as a group (not necessarily including all such insecticides). Group 2B (Possibly carcinogenic to humans) includes 16 pesticidal compounds or groups of compounds: aramite, chroldane, chlorophenoxy herbidides, chlorothalonil, dichloro-diphenyl-trichloroethane (DDT), dibromochloropropane (DBCP), *para*-dichlorobenzene, dichlorvos, heptachlor, hexachlorobenzene, lindane, mirex, nitrofen, sodium *ortho*-phenylphenate and toxaphene (www.iarc.fr; Siemiatycki et al. 2004). The classification concerns all types of cancer and thus extends beyond childhood leukemia.

Table 7. Pesticides evaluated by the International Agency for Research on Cancer (IARC) as carcinogenic (group 1), probably carcinogenic (group 2A) and possible carcinogenic (group 2B) to humans.

IARC category/ Pesticide name	Status in Costa Rica	Biocidal category/chemical group
Group 1		
Ethylene oxide	Sterilizing agent only	Insecticide, sterilizing agent
Chromium (VI) containing wood preservatives	Not used	Fungicides, insecticides.
Arsenical pesticides	Banned 1990[1]	Herbicides, fungicides, insecticides /arsenates and arsenic acids
TCDD[2]	Not used	Herbicides, waste incineration
Group 2A		
Captafol	Banned 1989	Fungicide/phtalimide chlorine
Ethyl dibromide	Banned 1988	Insecticide/aliphatic bromide
Nonarsenical insecticides[3]	In use	A large number of insecticides
Group 2B		
Aramite	Not used	Insecticide/phenoxymethyl esther
Chroldane	Banned 1991	Insecticide/organochlorine
Chlorophenoxy herbicides 2,4,5 T	Banned 1987	Herbicide/ phenoxyacetic acid
Chlorothalonil	In use	Fungicide/ benzonitril

Table 7. Pesticides evaluated by the International Agency for Research on Cancer (Cont.)

IARC category/ Pesticide name	Status in Costa Rica	Biocidal category/chemical group
DDT[4]	Banned 1988	Insecticide/organochlorine
DBCP[5]	Banned 1988	Nematocide/aliphatic, bromide chlorine
para-dichlorobenzene	Banned 1988	Fumigant/ benzene chlorine
Dichlorvos	In use	Insecticide/organophosphate
Heptachlor	Banned 1991	Insecticide/organochlorine
Hexachlorobenzene (lindane)	Banned 1996	Insecticide/organochlorine
Mirex	Banned 1999	Insecticide/organochlorine
Nitrofen	Banned 1988	Herbicide/nitrophenyl ether chlorine
Sodium *ortho*-phenylphenate	Not used	Fungicide, desinfectant/unclassified
Toxaphene	Banned 1988	Insecticide/organochlorine

1= Some pesticides were banned in the 1990s such as lead arsenate, but the herbicide methyl arsenic acid is still in use. 2= Tetrachlorodibenzodioxin. 3= IARC evaluation applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group. 4= Dichloro-diphenyl-trichloroethane. 5= Dibromochloropropane.

Table 7 shows the use status for the above mentioned pesticides in Costa Rica. Chlorothalonil and dichlorvos (both in Group 2B) are still in use in the country.

More generally, and also considering the inconclusive knowledge about cancer risks associated with most pesticidal compounds, including risk of childhood leukemia, one might wish to encourage targeting regulation at the most toxic pesticides, both acute and chronic toxicity considered, applying precautionary principle in the absence of sufficient health hazard data. Furthermore, the most efficient health promotion and disease prevention is often based on reduction or banning of exposures and agents rather than preventing their single health effects such as cancer only.

Finally, we want to state that our objectives in this study have been fulfilled. The results drawn from this study are being distributed among scientists, and will also be divulgated among the general population.

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Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006. (Papers from 1978-1998 adapted and modified from Zahm & Ward 1998). NEC: number of exposed cases. Total: total leukemia. ALL: acute lymphocytic leukemia. CML: chronic myeloid leukemia. NA: not applicable. NS: not significant ($p<0.05$). GIS: geographical information system

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case report (Acute Stem cell)	Infante et al. 1978 (US)	1	Chlordane	Annual house treatment	1	Case a girl of 9 years	
Case report (ALL)	Reeves et al. 1981 (US)	1	Dichlorvos, propoxur	Used in home 30 times, 1 day	1	Case a girl of 9 years	Reports on 8 children with bone marrow failure shortly after exposure to pesticides at home.
Case report (CML, ALL)	Reeves et al. 1982 (US)	13	Dichlorvos, propoxur	Matress sprayed twice/week, most of life	13		
Case-control (Total)	Hemminki et at. 1981 (Finland)	319	Paternal occupation as farmer	Pregnancy	156	1.3 NS	Paternal occupations collected from maternity welfare centers
Case-control (Total)	Gold et at. 1982 (US)	43	Paternal occupation as farmer	Before birth	2	vs 0 controls	Positive for pesticides, significance unknown
				Childhood	2	vs 0 controls	Positive for pesticides, significance unknown
Case-control (Total)	VanSteensel-Mol et al. 1985 (The Nederlands)	519	Maternal occupation in agriculture	Pregnancy	3	0.4 (0.1-1.7)	Relationship between prenatal chemical exposure in general and childhood leukemias. More parents of controls worked in agriculture.
				1 year < diagnosis	3	0.4 (0.1-1.3)	
			Maternal pesticide exposure	Pregnancy	4	0.7 (0.2-2.5)	
			Paternal occupation in agriculture	Pregnancy	35	0.9 (0.5-1.5)	
				1 year < diagnosis	32	0.9 (0.5-1.5)	
			Paternal pesticide exposure	Pregnancy	36	1.0 (0.6-1.7)	
Case-control (Total)	Lowengart et al. 1987 (US)	123	Parental pesticide use in home	Pregnancy and (mother only) nursing	19	3.8 (1.4-13.0)	
			maternal		13	3.2 p =0.02	
			paternal		12	4.0 p =0.02	
			Parental pesticide use in garden		13	6.5 (1.5-59.3)	
			maternal		9	9.0 p =0.02	
			paternal		5	5.0 p =0.07	
Case-control (Total)	Laval & Tuyns. 1988 (France)	201	Parental occupational exp to pesticides	Ever	12	vs 3 controls	
Case-control (Total, ALL, ANLL)	Shu et al. 1988 (China)	309	Occupation in agriculture:	Pregnancy			
			maternal (total)		12	2.3 (0.9-6.3)	
			maternal (ALL)		6	1.8 (0.6-5.4)	
			maternal (ANLL)		4	1.6 (0.4-6.3)	
			paternal (total leucemia)		2	0.3 (0.1-1.6)	
			Occupational pesticide exposure				
			maternal (total)		12	2.6 (0.8-9.1)	
			maternal (ALL)		7	3.5 (1.1-11.2)	
			maternal (ANLL)		3	2.4 (0.5-11.0)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (ANLL)	Buckley et al. 1989 (US)	204	Occupational pesticide exposure				
			paternal	Ever (+1000 days)	17	2.7 (1.0-7.0)	
				Before pregnancy	NA	1.7 NS	
				During pregnancy	NA	1.9 NS	
				After pregnancy	NA	1.8 NS	
			maternal	Ever (+1000 days)	7	vs 0 controls	
				Before pregnancy	NA	3.0 NS	
				During pregnancy	NA	6.0 NS	
				After pregnancy	NA	7.0 NS	
			Household pesticide exposure				
			maternal	<1/week	50	1.4 (0.8-2)	
				1-2/week	12	0.9 (0.4-2.1)	
				Most days	8	vs 0 controls; p(trend) 0.05	
			child	<1/week	46	1.8 (1.0-3.0)	
				1-2/week	13	2.0 (0.8-5.0)	
				Most days	8	3.5 (0.9-13.8); p(trend) 0.04	
Case-control (Total)	Gardner et al. 1990 (UK)	52	Paternal occupation as farmer	Birth	5	2.6 (0.8-9.0)	
Case-control (ALL)	Magnani et al. 1990 (Italy)	142	Paternal occupation as farmer	Before birth	4	1.8 (0.5-6.5)	
				Birth to diagnosis	5	5.6 (1.3-24.3)	
Case-control (ALL)	Infante-Rivard et al. 1991 (Spain)	128	Maternal occupation in agriculture	Pregnancy	9	1.8 (0.6-6.4)	
			Maternal insecticide exposure		7	1.4 (0.4-4.4)	
Case-control (ALL AML)	Schwartzbaum et al. 1991 (US)	522 (ALL)	Parental gardening with pesticides	Birth to diagnosis	NA	1.3 p =0.38	
		107(ANLL)			NA	0.9 p =0.38	
Case-control (ALL AML)	Scheele et al. 1992 (Germany)	35 ALL	Bone marrow levels of DDT[5]/DDE[6], HCB[7], HCH[8], dieldrin	At diagnosis	37	Nonpositive findings	
		3 AML					
Case-control (Total)	Deschamps et al. 1993 (Canada)	15	Pesticide sprayed in nearby parks, mosquito control, census data	Childhood	15	Nonpositive findings	
Case-control (ALL, Other leukemias)	Roman et al. 1993 (UK)	39 ALL	Paternal occupation in agriculture	Birth	11	1.1 (0.1-5.9)	
		11 Other		At interview	15	0.8 (0.1-3.3)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (Total)	Leiss & Savitz, 1995 (US)	NA	Pest strips	Last 3 month pregnancy	21	3.0 (1.6-57)	
				Birth-2 years < diagnosis	21	1.7 (1.2-24)	
				2 y < diagnosis to diagnosis	18	2.6 (1.7-3.9)	
			House extermination	Last 3 month pregnancy	4	0.4 (0.1-1.2)	
				Birth-2 years < diagnosis	6	0.3 (0.1-0.8)	
				2 y < diagnosis to diagnosis	7	0.9 (0.5-1.4)	
			Yard pesticide treatment	Last 3 month pregnancy	27	1.1 (0.6-1.9)	
				Birth-2 years < diagnosis	36	0.9 (0.5-1.8)	
				2 y < diagnosis to diagnosis	33	1.1 (0.8-1.5)	
Cohort[2] (ALL AML, Other)	Kristensen et al. 1996 (Norway)	181	Parental agricultural work.	Before birth	113 Tot	1.0 (0.8-1.2)	Exposures were estimated by crude proxies with possible misclassification (expenditures on pesticides and spraying equipment)
			Census pesticide expenditures		52 Acute	1.1 (0.8-1.5)	
					29 ALL	1.0 (0.9-1.7)	
					12 AML	1.4 (0.6-2.9)	
					11 Other	0.9 (0.4-1.9)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (Total)	Meinert et al. 1996 (Germany)	173	Occupational exposure to pesticides				Matched by year of birth and gender. No information about significance of OR. Possible misclassification of exposure due to crude exposure assessment.
			father	Ever	9	1.19 NS	
				Year before pregnancy	9	1.19 NS	
				Pregnancy	5	1.76 NS	
				Childhood	9	1.33 NS	
			mother	Ever	4	1.58 NS	
				Year before pregnancy	2	1.58 NS	
				Pregnancy	2		
				Childhood	4		
			either parent	Ever	12	1.53 NS	
				Year before pregnancy	11	1.53 NS	
				Pregnancy	7	2.22 NS	
				Childhood	12	1.9 NS	
			Any pesticide facility use	Ever	27	2.5 (1.1-5.4)	
			garden	2 year before birth	20	2.5 (1.0-6.1)	
			farm		7	1.6 (0.4-6.9)	
			House exterminator by any		37	0.8 NS	
			House exterminator by pest controller		3	1.1 NS	
Case-control (ALL)	Infante-Rivard & Sinnett, 1999 (Canada)	175	Paternal occupational exposures to pesticides	Preconceptional	150		Speculation of mechanisms: genomic imprinting, involving paternal genome, through alteration of DNA methylation. Possitive association with fungicides and fertilizers
			total pesticides		66	1.6 (1.1-2.4)	
			fungicides		15	5.1 (1.5-17.8)	
			insecticides		50	1.4 (0.9-2.2)	
			herbicides		19	2.1 (0.9-4.6)	
			Fertilizers		25	2.9 (1.3-6.6)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (ALL)	Infante-Rivard et al.1999 (Canada)	491	Home exposure to insecticide/rodenticide				Results similar for *in utero* and the postnatal periods. Interviews were not blinded to case or control status for ethical reasons.
			cockroaches, ants, flies	pregnancy	168	1.8 (1.3-2.4)	
			moths		45	2.5 (1.4-4.3)	
			insects		96	1.6 (1.1-2.3)	
			cockroaches, ants, flies	childhood	212	1.4 (1.1-1.8)	
			moths		50	2.1 (1.3-3.5)	
			insects		137	3.0 (1.4-2.8)	
			Pesticide use in garden	pregnancy			
			herbicides		118	1.8 (1.3-2.6)	
			plant insecticides		78	2.0 (1.3-2.9)	
			products for trees		63	1.7 (1.1-2.6)	
			herbicides	childhood	178	1.4 (1.1-1.9)	
			plant insecticides		128	1.8 (1.3-2.5)	
			products for trees		99	1.4 (1.0-2.0)	
Case-control (Total)	Meinert et al. 2000 (Germany)	1184	Parental occupational exposure to pesticides (interview)				Risks are higher before and during pregnancy as compared to after pregnancy.
			paternal	Ever	68	1.6 (1.1-2.3)	Maternal risks are higher than paternals
				Year before pregnancy	62	1.5 (1.1-2.2)	Pesticides on farms are more associated with leukemia in children than pesticides on gardens. Possible dose-response-like pattern.
				Pregnancy	57	1.6 (1.1-2.3)	
				Childhood	49	1.3 (0.9-1.9)	
			maternal	Ever	24	2.5 (1.3-4.7)	
				Year before pregnancy	19	2.1 (1.1-4.2)	
				Pregnancy	15	3.6 (1.5-8.8)	
				Childhood	12	2.5 (1.0-6.4)	
			Pesticide use				
			in garden		164	1.0 (0.8-1.2)	
			on farm		54	1.5 (1.0-2.2)	
			Use of household insecticides:	Times per year			
			by parent	-<1	971	1	
				-1	43	1.0 (0.7-1.5)	
				->1:	90	1.2 (0.9-1.6)	
				-6-10	18	1.3 (0.7-2.4)	
				->10	22	1.8 (1.0-3.3)	
			by pest controller		25	1.3 (0.8-2.3)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (Total and ALL)	Petridou et al. 2000 (Greece)	153	Maternal ¨non-negligible¨ exposure to pesticides	Prenatal	12	3.6 (1.2-10.8) (Total)	Associations with exposure to pesticides
					10	2.9 (0.9-9.9) (ALL)	
Cohort[3]	Feychting et al. 2001 (Sweden)	161	Father's occupation (Registries)	Birth to 15 years old or 1993.	5	0.9 (0.4-2.2)	Fathers' occupation linked to JEM. Major weakness is the exposure assessment. In Sweden pesticide use is restricted and regulated by authorities.
Ecologic	Reynolds et al. 2002 (US)	2443	Agricultural pesticide use density, individual pesticides			RR	Risk association with agricultural use of propargite
			Propargite	<1 lb/mi^2	2191	1	
				1-74 percentile	191	0.9 (0.8-1.1)	
				75-89 percentile	26	0.7 (0.5-1.1	
				>=90 percentile	35	1.5 (1.0-2.1)	
			Mehyl bromide	<1 lb/mi^2	1981	1	
				1-74 percentile	335	1.0 (0.9-1.2)	
				75-89 percentile	73	1.1 (0.9-1.5)	
				>=90 percentile	54	1.1 (0.9-1.5)	
			Metam sodium	<1 lb/mi^2	2293	1	
				1-74 percentile	116	1.0 (0.8-1.2)	
				75-89 percentile	19	0.9 (0.5-1.5)	
				>=90 percentile	15	0.9 (0.5-1.7)	
			Trifluralin	<1 lb/mi^2	2286	1	
				1-74 percentile	127	0.9 (0.8-1.2)	
				75-89 percentile	18	0.7 (0.4-1.3)	
				>=90 percentile	12	0.9 (0.5-1.6)	
			Simazine	<1 lb/mi^2	2198	1	
				1-74 percentile	179	0.9 (0.8-1.1)	
				75-89 percentile	47	1.4 (0.9-2.0)	
				>=90 percentile	19	0.8 (0.5-1.4)	
			Dicofol	<1 lb/mi^2	2283	1	
				1-74 percentile	130	1.0 (0.8-1.2)	
				75-89 percentile	18	0.7 (0.4-1.2)	
				>=90 percentile	12	0.7 (0.4-1.3)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
	Reynolds et al. 2002 (US) Cont.		Chlorothalonil	<1 lb/mi^2	2119	1	
				1-74 percentile	243	1.0 (0.9-1.2)	
				75-89 percentile	40	0.9 (0.6-1.3)	
				>=90 percentile	41	1.3 (0.9-1.8)	
			Agricultural pesticide use density, toxicological groups				
			Class B carcinogens	<1 lb/mi^2	1834	1	
				1-74 percentile	455	0.9 (0.8-1.0)	
				75-89 percentile	95	1.1) 0.8-1.3)	
				>=90 percentile	59	0.9 (0.6-1.2)	
			Class C carcinogens	<1 lb/mi^2	1639	1	
				1-74 percentile	620	1.0 (0.9-1.1)	
				75-89 percentile	114	0.9 (0.7-1.2)	
				>=90 percentile	70	0.9 (0.7-1.2)	
			Genotoxins	<1 lb/mi^2	1495	1	
				1-74 percentile	691	0.9 (0.8-1.0)	
				75-89 percentile	145	1.0 (0.8-1.2)	
				>=90 percentile	112	1.1 (0.9-1.4)	
			Developmental and reproductive toxins	<1 lb/mi^2	1590	1	
				1-74 percentile	616	0.9 (0.8-1.0)	
				75-89 percentile	135	1.0 (0.8-1.3)	
				>=90 percentile	102	1.1 (0.9-1.4)	
			Agricultural pesticide use density, chemical groups				
			Organochlorines	<1 lb/mi^2	2200	1	
				1-74 percentile	196	1.1 (0.9-1.3)	
				75-89 percentile	31	0.9 (0.6-1.3)	
				>=90 percentile	16	0.7 (0.4-1.2)	
			Organophosphates	<1 lb/mi^2	1662	1	
				1-74 percentile	601	1.0 (0.9-1)	
				75-89 percentile	109	0.9 (0.7-1.2)	
				>=90 percentile	71	0.91 (0.7-1.2)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
	Reynolds et al. 2002 (US) Cont		Carbamates	<1 lb/mi^2	1917	1	
				1-74 percentile	411	1.0 (0.9-1.1)	
				75-89 percentile	60	0.8 (0.59-1.07)	
				>=90 percentile	55	1.0 (0.7-1.4)	
			Dithiocarbamates	<1 lb/mi^2	2035	1	
				1-74 percentile	313	1.0 (0.9-1.2)	
				75-89 percentile	58	0.9 (0.7-1.2)	
				>=90 percentile	37	0.9 (0.6-1.3)	
Case-control (Total and ALL)	Ma et al. 2002 (US)	162 (Total)	Household pesticide use				Insecticides exposures early in life appeared more significant than later exposures. More frequent exposures to insecticides was associated with higher risks. Exposure to indoor pesticides was associated with an increased risk.
			Profesional pest control	3 months < pregnancy	16	1.7 (0.7-3.9)	
				During pregnancy	22	2.2 (1.0-4.8)	
				1st year of life	25	2.3 (1.1-4.9)	
				2nd year of life	31	3.6 (1.6-8.3)	
				3rd year of life	23	2.2 (1.0-4.7)	
				All time windows	39	2.8 (1.4-5.7)	
			Insecticides	3 months < pregnancy	63	1.8 (1.1-3.1)	
				During pregnancy	79	2.1 (1.3-3.5)	
				1st year of life	90	1.7 (1.0-2.9)	
				2nd year of life	90	1.6 (1.0-2.7)	
				3rd year of life	76	1.2 (0.7-2.1)	
				All time windows	93	2.1 (1.1-4.3)	
			Flea control products	3 months < pregnancy	26	0.9 (0.5-1.7)	
				During pregnancy	27	0.8 (0.4-1.4)	
				1st year of life	34	0.8 (0.5-1.4)	
				2nd year of life	35	0.9 (0.5-1.5)	
				3rd year of life	26	0.8 (0.4-1.4)	
				All time windows	40	0.9 (0.5-1.6)	
			Herbicides	3 months < pregnancy	31	1.8 (0.9-3.5)	
				During pregnancy	34	1.6 (0.9-3.0)	
				1st year of life	35	0.7 (0.4-1.2)	
				2nd year of life	40	1.1 (0.7-2.0)	
				3rd year of life	33	1.1 (0.6-2.1)	
				All time windows	38	1.0 (0.6-1.8)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
	Ma et al. 2002 (US) Cont.		Indoor pesticides	During pregnancy	NA	2.2 (1.3-3.6)	
				All time windows	89	1.8 (1.0-3.4)	
		NA (ALL)	Profesional pest control	3 months < pregnancy	15	1.9 (0.7-4.7)	
				During pregnancy	20	2.3 (0.3-5.4)	
				1st year of life	22	2.1 (1.0-4.7)	
				2nd year of life	29	3.3 (1.4-7.7)	
				3rd year of life	21	2.1 (1.0-4.7)	
				All time windows	36	2.6 (1.2-5.4)	
			Insecticides	3 months < pregnancy	53	1.7 (1.0-3.1)	
				During pregnancy	68	2.3 (1.3-4.0)	
				1st year of life	75	1.7 (1.0-2.9)	
				2nd year of life	78	1.7 (1.0-2.9)	
				3rd year of life	65	1.1 (0.6-2.1)	
				All time windows	80	2.2 (1.0-4.6)	
			Flea control products	3 months < pregnancy	22	0.8 (0.4-1.6)	
				During pregnancy	22	0.7 (0.4-1.4)	
				1st year of life	31	0.9 (0.5-1.6)	
				2nd year of life	32	1.0 (1.5-1.8)	
				3rd year of life	24	0.9 (0.5-1.7)	
				All time windows	36	1.0 (0.5-1.8)	
			Herbicides	3 months < pregnancy	24	1.6 (0.8-3.3)	
				During pregnancy	30	1.8 (0.9-3.5)	
				1st year of life	32	0.8 (0.4-1.4)	
				2nd year of life	36	1.1 (0.6-2.0)	
				3rd year of life	31	1.2 (0.6-2.3)	
				All time windows	35	1.0 (0.6-1.8)	
Cohort[4] (All cancer sites)	Rodvall et al. 2003 (Sweden)	51 (all cases)	Licensed pesticide applicators		8	0.4 (0.2-0.9)	Reduced risk for leukemia. Offsprings of farmers in healthier environment with regard to air pollution, exhausts, smoking, diet, drinking water, less stress, more physical activity, etc.
Nested case-control	Flower et al. 2004 (US)	9	Parent's pesticide mixing and /or application (self report).	Percent of time mixing of applying pesticides		SIR 0.9 (0.5-1.7)	Total childhood cancer. Registered private applicators were assessed through questionnaires.

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (Total)	Reynolds et al. 2005 (US)	2189	Agricultural pesticide use density, individual pesticides. (GIS[5] and registries)	Pregnancy			Mother's address at birth taken as surrogate for potential exposure during pregnancy.
			Propargite	<1 lb/mi^2	784	1.0	Suggested risk association with insecticide dicofol, fumigant metam sodium. Also with carbamates
				<50 percentile	21	0.9 (0.5-1.5)	
				>50 percentile	32	1.0 (0.6-1.5)	
			Methyl bromide	<1 lb/mi^2	756		
				<50 percentile	44	0.9 (0.6-1.4)	
				>50 percentile	37	0.9 (0.6-1.3)	
			Metam sodium	<1 lb/mi^2	813	1.0	
				<50 percentile	8	0.8 (0.4-1.9)	
				>50 percentile	16	2.1 (1.0-4.2)	
			Trifluralin	<1 lb/mi^2	803	1.0	
				<50 percentile	16	1.1 (0.6-1.9)	
				>50 percentile	18	0.9 (0.5-1.6)	
			Simazine	<1 lb/mi^2	775	1.0	
				<50 percentile	31	1.2 (0.7-1.9)	
				>50 percentile	31	1.3 (0.8-2.0)	
			Dicofol	<1 lb/mi^2	802	1.0	
				<50 percentile	10	0.7 (0.4-1.5)	
				>50 percentile	25	1.8 (1.0-3.2)	
			Chlorothalonil	<1 lb/mi^2	765	1.0	
				<50 percentile	32	1.2 (0.8-1.9)	
				>50 percentile	40	1.3 (0.9-2.0)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
	Reynolds et al. 2005 (US) Cont.		Toxicological groups				
			Class B carcinogens	<1 lb/mi^2	643	1.0	
				<50 percentile	89	1.1 (0.8-1.5)	
				>50 percentile	105	1.2 (0.9-1.5)	
			Class C carcinogens	<1 lb/mi^2	582	1.0	
				<50 percentile	113	1.2 (0.9-1.5)	
				>50 percentile	142	1.3 (0.9-1.6)	
			Genotoxins	<1 lb/mi^2	563	1.0	
				<50 percentile	128	1.0 (0.8-1.3)	
				>50 percentile	146	1.1 (0.8-1.3)	
			Development and reproductive toxins	<1 lb/mi^2	585	1.0	
				<50 percentile	130	1.2 (0.9-1.6)	
				>50 percentile	122	1.0 (0.8-1.3)	
			Chemical groups				
			Organochlorines	<1 lb/mi^2	778	1.0	
				<50 percentile	30	1.1 (0.7-1.8)	
				>50 percentile	29	1.3 (0.8-2.1)	
			Organophosphates	<1 lb/mi^2	598	1.0	
				<50 percentile	102	1.0 (0.8-1.3)	
				>50 percentile	137	1.2 (0.9-1.6)	
			Carbamates	<1 lb/mi^2	678	1.0	
				<50 percentile	85	1.4 (1.0-1.9)	
				>50 percentile	74	1.1 (0.8-1.5)	
			Dithiocarbamates	<1 lb/mi^2	730	1.0	
				<50 percentile	51	1.0 (0.7-1.5)	
				>50 percentile	56	1.0 (0.7-1.4)	

Appendix 1. International comparison studies of childhood leukemia and pesticide exposure published from 1978-2006.

Study design (leukemia output)	Reference (country)	Cases	Exposure	Timing/ details of exposure	NEC	Risk estimate[1]	Comments
Case-control (Total)	Menegaux et al. 2006 (France)	280	Home insecticide use (interview)				Home pesticides' exposure risks are consistent with other studies. Negative association with parental occupational exposure, due to low NEC Association with garden pest use is less consistent with other studies. Exposures to insecticides and fungicides during childhood. Association with history of pediculosis, treatment with insecticidal shampoo and pyrethroids.
			during pregnancy	Never	188	1.0 (ref)	
				Ever	92	1.8 (1.2-2.8)	
			during childhood	Never	164	1.0 (ref)	
				ever	111	1.7 (1.1-2.4)	
			Garden pesticide use				
			during pregnancy	Never	252	1.0 (ref)	
				Ever	14	2.5 (0.8-7.2)	
				Insecticide	9	1.9 (0.6-6.5)	
				Herbicide	6	5.9 (0.7-52)	
				fungicide	3	-	
			during childhood	Never	191	1.0 (ref)	
				Ever	70	1.7 (1.1-2.7)	
				Insecticide	47	2.4 (1.3-4.3)	
				Herbicide	40	1.4 (0.8-2.4)	
				fungicide	17	2.5 (1.0-6.2)	
			Pediculosis				
			pediculosis	Never	180	1.0 (ref)	
				Ever	98	1.6 (1.0-2.6)	
				Once	51	1.5 (0.9-2.5)	
				Twice or more	47	1.9 (1.1-3.3)	
			pediculosis treatment	Never	179	1.0 (ref)	
				Insecticidal shampoo	70	1.9 (1.1-3.2)	
				Other (vinegar, etc.)	1	0.3 (0.03-2.9)	
				Pyrethroid	65	2.0 (1.1-3.4)	
				Pyrethroid only	59	2.2 (1.2-3.8)	
				Lindane	6	2.1 (0.5-8.7)	
				Malathion	5	0.7 (0.2-2.4)	
Case-control (ALL)	Abadi-Korek et al. 2006 (Israel)	112	Paternal and maternal occupational pesticide exposure. Jobtitles as surrogates for exposure.	Exposure to at least 6 months' before date of diagnosis.	57	2.4 (1.1-5.0)	All occupations were analyzed together by time-windows during gestation. Pesticides in general

1- Case-control risk estimates are odds ratios (OR). 95% CI in parentheses. 2- Risk estimate was incidence rate ratio. 3- Risk estimate was relative risk. 4- Risk estimate was standardized incidence rate. 5- Dichlorodiphenyltrichloroethane. 6- Dichlorodiphenyldichloroethylene. 7- Hexachlorobenzene; 8- Hexachlorocyclohe