Pesticides and childhood cancer

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Plan

- Brief review of epidemiological findings for childhood leukemia and brain cancer
  - Residential and parental occupational pesticide exposures
- Some new results for parental occupational exposure for ALL
- Brief comments on studies considering genetic variants as modifiers of the effects of pesticides
- Plausibility of overall results from epi studies
  - Biological plausibility
  - Regulatory agency decisions
  - Alternative explanations

Classification of pesticides

- Based on Target Pest
  - Algae- Algicide
  - Bacteria- Bactericide
  - Birds- Avicide
  - Fish- Piscicide
  - Fungi- Fungicide
  - Insects- Insecticide
  - Mites- Miticide/Acaricide
  - Mollusks- Molluscicide
  - Nematodes- Nematicide
  - Rodents- Rodenticide
  - Spiders- Arachnidcide
  - Trees- Arboricide
  - Weeds- Herbicide

Classification of pesticides

- Based on Chemical Nature
  - *Inorganic*: do not contain carbon (Lead arsenate, Paris Green, Sulfur, Zinc Phosphate)
  - Synthetic *Organic*
    - a. Chlorinated hydrocarbon
    - b. Organophosphate
    - c. Carbamate
    - d. Synthetic Pyrethroid
    - e. New Chemicals (Neonicotinoid, Pyrrole, Phenylpyrazole)
  - *Biorational* derived from various biological sources (Pheromone, Insect Growth Regulator, Microbial, Naturalyte, Macrolactone-Avermectin, Botanical)

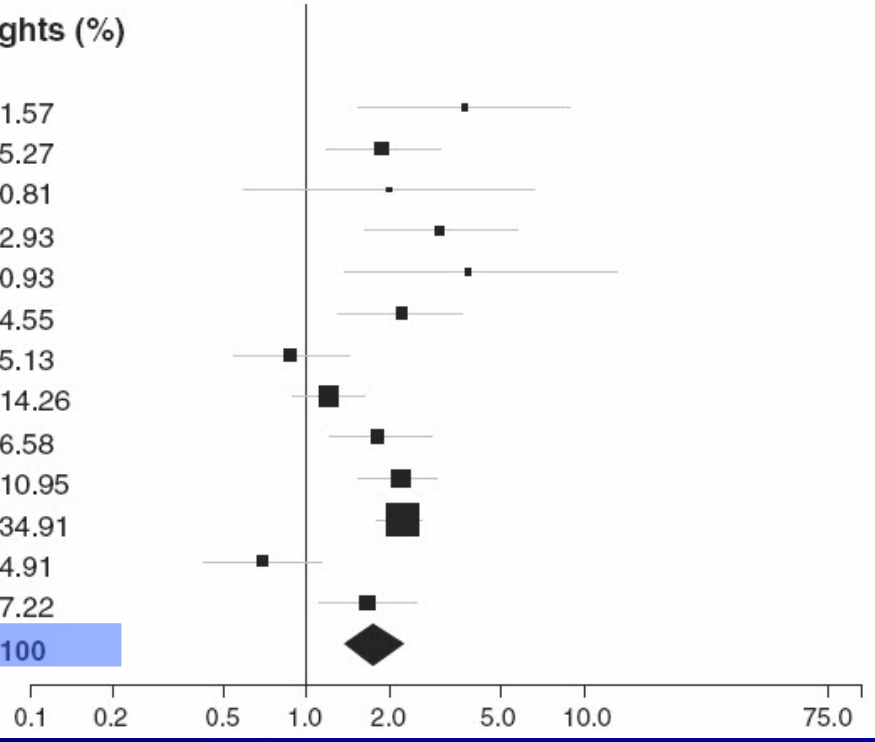
Results for leukemia meta-analyses (MA) for residential exposure

- MA by Van Maele-Fabry et al., 2011
  - The MA relates its results to those from 3 previous comprehensive narrative reviews
    - Daniels et al. 1999
    - Zahm & Ward 1998
    - Infante-Rivard & Weichenthal 2007
  - This MA found results in agreement with the conclusions of the previous
  - Time window definitions for all results/studies are described; a few broad inclusive categories are used in the analyses
- MA by Turner et al., 2009

Results for leukemia (Van Maele-Fabry)

parental E during pregnancy and/or before pregnancy & child postnatal, indoor and outdoor residential exposure

Study	RR (95% C.I.)	Weights (%)
Alexander et al., 2001	3.67 (1.54-8.74)	1.57
Buckley et al., 1989	1.85 (1.16-2.99)	5.27
Infante-Rivard et al., 1999	1.98 (0.59-6.62)	0.81
Leiss and Savitz, 1995	3 (1.6-5.7)	2.93
Lowengart et al., 1987	3.8 (1.37-13.02)	0.93
Ma et al., 2002	2.2 (1.3-3.6)	4.55
Meinert et al., 1996	0.87 (0.54-1.41)	5.13
Meinert et al., 2000	1.2 (0.9-1.6)	14.26
Menegaux et al., 2006	1.8 (1.2-2.8)	6.58
Pombo-de-Oliveira et al., 2006	2.18 (1.53-2.95)	10.95
Rudant et al., 2007	2.2 (1.8-2.6)	34.91
Spix et al., 2009	0.69 (0.42-1.12)	4.91
Urayama et al., 1985	1.65 (1.1-2.47)	7.22
Total	1.74 (1.37-2.21)	100



Results for leukemia (Van Maele-Fabry)

residential exposure

	N.	mRR	95% CI
Residential pesticide exposure†			
A. All studies (A.1)	13	1.74	1.37–2.21
B. Exposure time windows			
(B.1) During pregnancy	9	**2.19**	1.92–2.50
(B.2) After pregnancy (childhood)	6	1.65	1.33–2.05
(B.3) Others	5	1.28	0.81–2.03
F. Leukaemia type			
ANLL			
(F.1) All studies	3	**2.30**	1.53–3.45
(F.2) Insecticides, pregnancy	2	**3.13**	1.45–6.75
ALL			
(F.3) All studies	5	**2.17**	1.83–2.56
Insecticides			
(F.4) All studies	5	**2.11**	1.80–2.48
(F.5) Pregnancy	4	**2.22**	1.87–2.64
(F.6) Childhood	2	**1.78**	1.12–2.84
Herbicides			
(F.7) All studies	3	1.47	0.98–2.2
(F.8) Pregnancy	3	**1.78**	1.41–2.24
(F.9) Childhood	2	1.14	0.67–1.95

Results for leukemia (Turner) residential exposure

- Preconceptional household use:
  - Indoor OR=1.53 (0.98-2.39)
  - Outdoor OR=1.69 (1.02-2.77)
- Exposures during pregnancy:
  - unspecified pesticides OR=1.54 (1.13–2.11)
  - insecticides OR=2.05 (1.80–2.32)
  - herbicides (OR=1.61 (1.20–2.16)
- Exposures during childhood
  - unspecified pesticides OR= 1.38 (1.12–1.70)
  - insecticides OR=1.61 (1.33–1.95)
  - herbicides (no association)

Results for leukemia residential exposure **definition issues** (from Turner et al.,)

- **Preconception**
  - 3 months before conception
  - 2 years before conception
  - 3 months before pregnancy to lactation
  - 2 years before birth to date of diagnosis/reference date
  - 1 year before pregnancy to reference date
- **Pregnancy**
  - 3 months before birth
  - Conception to birth
  - 1 month before pregnancy to birth
  - Conception to lactation (maternal)
  - 1 month before pregnancy, pregnancy, and lactation
  - 3 months before pregnancy to lactation
  - 2 years before birth to date of diagnosis/reference date
  - Year of birth to diagnosis/reference date

Results for leukemia residential exposure definition issues (from Turner et al.)

- **Childhood**
  - End of lactation to date of diagnosis/reference date
  - Birth to date of diagnosis/reference date
  - Birth to 2 years before diagnosis, and 2 years before diagnosis to diagnosis
  - Years 1, 2, and 3 after birth
  - Onset of disease
  - Birth to 6 months, and 7 months to date of diagnosis/reference date
  - Pregnancy and childhood, paternal
  - 2 years before birth to date of diagnosis/reference date
  - Year of birth to diagnosis/reference date
  - 1 year before pregnancy to reference date

Results for leukemia parental occupational exposures

- Based on two meta-analyses:
  - Van Maele-Fabry et al., 2010
    - Stipulated use of pesticides
    - Job title (agriculture/farm)
  - Wigle et al., 2009

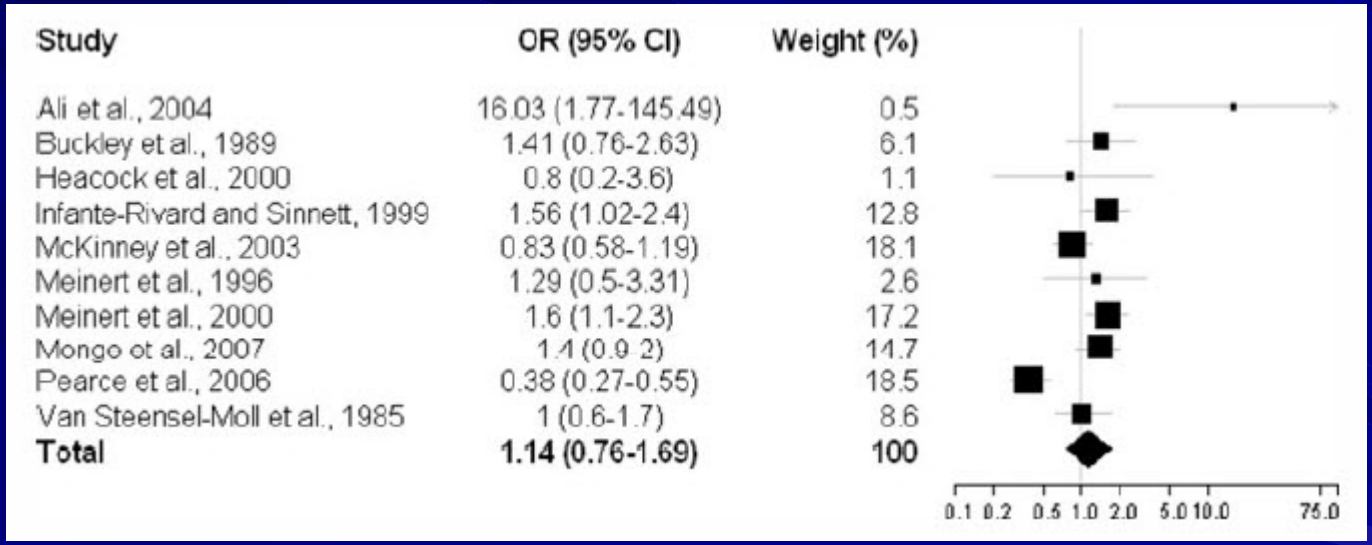
Results for leukemia (Van Maele-Fabry) (paternal occupational exposure)

Paternal			
(A.1) Pesticides all studies	10b	1.14	0.76–1.69
Windows of exposure			
(A.2) before pregnancy	5	*1.41*	1.15–1.74
(A.3) during pregnancy	4a	*1.36*	1.08–1.72
(A.4) after pregnancy	3	1.25	0.95–1.65
(A.5) before + during pregnancy + at birth	3b	0.83	0.33–2.07
(A.6) any time/unspecified/ever	6	*1.49*	1.18–1.89
Leukaemia type			
(A.7) ALL	3	1.09	0.75–1.60
(A.8) ANLL	2	0.73	0.19–2.76
Biocide category			
(A.9) insecticides	2	*1.39*	1.02–1.90
(A.10) herbicides	2	*1.51*	1.06–2.16
(A.11) fungicides	4	*2.65*	1.05–6.67

Results for leukemia (Van Maele-Fabry) (maternal occupational exposure)

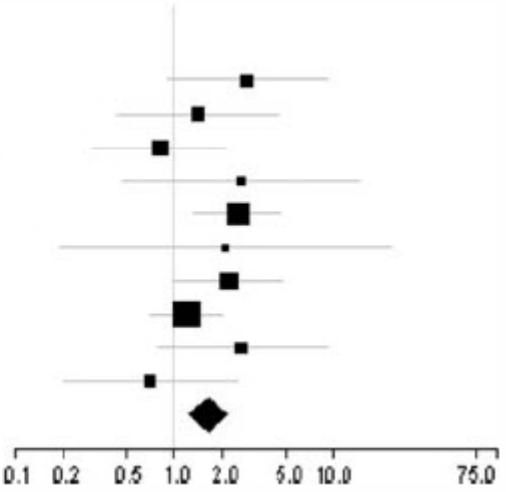
Maternal			
(A.12) Pesticides all studies	10	*1.62*	1.22–2.16
Windows of exposure			
(A.13) before pregnancy	3	*2.24*	1.34–3.72
(A.14) during pregnancy	8a	*2.00*	1.11–3.62
(A.15) after pregnancy	3	*2.25*	1.21–4.20
(A.16) any time/ever	4	*2.45*	1.58–3.81
Leukaemia type			
(A.17) ALL	4	1.34	0.70–2.59
(A.18) ANLL	2	*2.68*	1.06–6.78
Biocide category			
(A.19) insecticides	2	2.11	0.97–4.62

Results for leukemia (Van Maele-Fabry) (paternal occupational exposure)



Results for leukemia (Van Maele-Fabry) (maternal occupational exposure)

Study	OR (95% CI)	Weight (%)
Buckley et al., 1989	2.85 (0.89-9.1)	6.1
Infante-Rivard et al., 1991	1.4 (0.44-4.41)	6.2
McKinney et al., 2003	0.81 (0.31-2.12)	9
Meinert et al., 1996	2.59 (0.47-14.3)	2.8
Meinert et al., 2000	2.5 (1.3-4.7)	20.1
Menegaux et al., 2006	2.07 (0.19-22.9)	1.4
Monge et al., 2007	2.2 (1-4.8)	13.5
Rudant et al., 2007	1.2 (0.7-2)	30.1
Shu et al., 1988	2.6 (0.8-9.1)	5.6
Van Steensel-Moll et al., 1985	0.7 (0.2-2.5)	5.2
Total	**1.62 (1.22-2.16)**	**100**

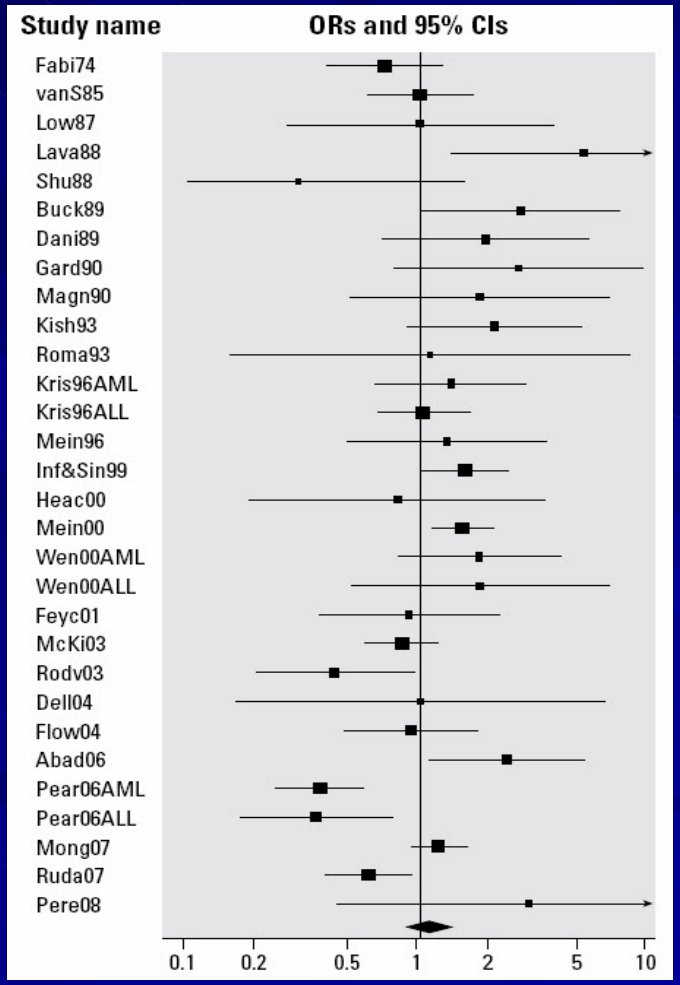


Summary (Van Maele-Fabry) parental occupational exposures

- Paternal
  - All pesticides; all leukemias; all periods
    - OR=1.14 (0.76-1.69)
  - Before conception (all leukemias; all pesticides)
    - OR=1.41 (1.15-1.74)
- Maternal:
  - All pesticides; all leukemias; all periods
    - OR=1.62 (1.22-2.16)
  - During pregnancy (all leukemias; all pesticides)
    - OR=2.00 (1.11-3.62)

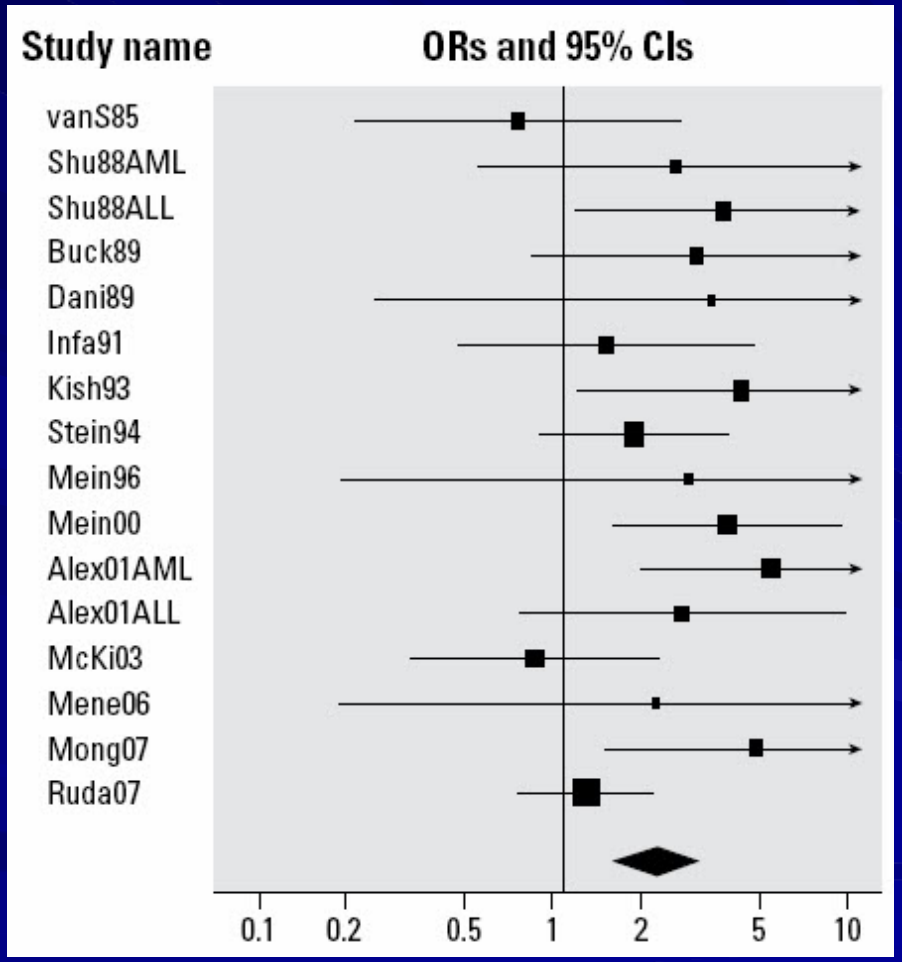
Results for leukemia (Wigle)

any paternal occupational exposure
(mainly 2y before conception but also during pregnancy)



Results for leukemia (Wigle)

(maternal occupational exposure (during pregnancy)



Results for leukemia (Wigle)

parental occupational exposure
(paternal includes before and during pregnancy)

Exposure (no. of risk estimates)[a]	Summary OR (95% CI)
Paternal occupational exposure	
Any pesticide exposure[c] (*n* = 30)	1.09 (0.88–1.34)
Unspecified pesticides only[d] (*n* = 26)	1.04 (0.83–1.31)
Prenatal maternal occupational exposure	
Any pesticide exposure (*n* = 16)	2.09 (1.51–2.88)
Unspecified pesticides only (*n* = 14)	2.16 (1.51–3.08)

Results for paternal occupational exposure definition issues (from Wigle et al.)

- Well-defined preconceptual window

- a) Preconceptual period <2 years
  - Occupational pesticide exposure during year before conception
  - Occupational pesticide exposure during 2 yr before conception
  - Occupational pesticide exposure during 1 yr before conception
  - Occupation in farming for 6+ months during 2 yr before conception

- b) Preconceptual exposure reasonably inferable
  - Occupation in farming at child's birth
  - Occupational pesticide exposure during pregnancy
  - Occupation in farming during pregnancy
  - Occupation in farming at child's birth
  - Job title with likely pesticide exposure 2-26 mos before child's birth
  - Agricultural chemical use during 1 yr before child's birth
  - Job title with likely pesticide exposure at child's birth

Results for paternal occupational exposure definition issues (from Wigle et al.)

- Ill-defined exposure window
  - Occupation in farming 1 yr before conception to 1 yr before diagnosis
  - Any occupational pesticide exposure 1 yr before birth to diagnosis
  - Any preconceptual agricultural pesticide use
  - Occupation in farming before child's birth
  - Occupational pesticide exposure during preconceptual period
  - Farmer licensed as pesticide applicator during preconceptual period
  - Parental occupational pesticide exposure; timing not stated
  - Occupation as farmer and record of pesticide purchasesd
  - Cumulative lifetime occupational chlorophenate exposure
  - Occupational herbicide exposure up to 15+ yrs before conception
  - Licensed as pesticide applicator up to 29 yr before child's birth
  - Job title with likely pesticide exposure before date of diagnosis

MA for all cancers (Vinson et al. 2011)

residential and parental occupational exposures

- Definitions:
  - studies from 1985-2009 (Searles Nilesen et al. 2010) is not included but reports mainly on GxE interactions)
  - prenatal exposure:
    - includes exposure before conception.
  - postnatal exposure of parents:
    - parents having either agricultural or non-agricultural occupations or using pesticides at home or in the garden, incuding use of professional pest control services (indoor or outdoor).
  - exposure classified as 'ever' corresponds to an unspecified period of exposure by authors
  - occupational exposure of parents refers to agricultural (farmers, farm workers) or non-agricultural occupations (chemical industry, pest controller).

Leukemia and brain cancer (Vinson et al 2011) all types of exposures

	Exposed person	Leukaemia	Brain
Prenatal exposure	Mother	25 (random) 1.48 (1.26 to 1.75) No bias	NS
	Father	18 (random) 1.32 (1.20 to 1.46) No bias	9 (fixed) 1.49 (1.23 to 1.79) No bias
	Father and mother	4 (fixed) 1.84 (1.39 to 2.44) No bias	5 (fixed) 1.37 (1.08 to 1.76) No bias
Postnatal exposure	Mother	3 (fixed) 2.12 (1.17 to 3.84)	ND
	Father	4 (fixed) 1.33 (1.07 to 1.66) No bias	2 (fixed) 1.66 (1.11 to 2.49)
	Father and mother	NS	ND
	Child	NS	21 (random) 1.16 (1.01 to 1.32)
'Ever'	Mother	ND	NS
	Father	NS	10 (fixed) 1.41 (1.11 to 1.79) No bias
	Father and Mother	NS	NS
	Child	2 (fixed) 1.85 (1.15 to 2.96) Bias	ND

Leukemia and brain cancer (Vinson et al 2011) all periods

	Occupational exposure of parents to pesticides		Parents' use of pesticides in the home or garden		Living in an active agricultural zone
	Father	Mother	Father	Mother	
Leukaemia					
Test for heterogeneity	Fixed	ND	Random	Random	NS
OR (95% CI)	1.37 (1.23 to 1.52)		1.26 (1.06 to 1.49)	1.56 (1.21 to 2.02)	
No of data	21		3	9	
Brain					
Test for heterogeneity	Fixed	NS	Fixed	NS	NS
OR (95% CI)	1.40 (1.20 to 1.62)		1.48 (1.22 to 1.80)		
No of data	11		13		

Leukemia and brain cancer (Vinson et al 2011)
all periods and both parents

Type of cancer	Herbicide	Insecticide	Fungicide
Leukaemia			
Test for heterogeneity	Fixed	Random	NS
OR (95% CI)	1.26 (1.14 to 1.39)	1.17 (1.03 to 1.33)	
No of data	20	45	
Brain			
Test for heterogeneity	Random	Fixed	Random
OR (95% CI)	1.31 (1.08 to 1.60)	1.18 (1.06 to 1.33)	1.32 (1.06 to 1.65)
No of data	16	24	15

Summary (Vinson) all leukemias; all types of exposures

- Mother (preconception and pregnancy)
  - OR=1.48 (1.26-1.75)
- Father (preconception and during pregnancy)
  - OR=1.32 (1.20-1.46)
- Postnatal exposure: child
  - OR (NS)

Summary of (selected) MA results from environmental epi studies

- **Leukemia**
- Preconception for fathers:
  - Occupational
    - 2/3 MA→+
- During pregnancy for mothers
  - Occupational
    - 2/3 MA→ +
  - Residential
    - 3/3 MA→+
- Child exposure post-natally
    - 2/3 MA→+

- **Brain cancer**
- Preconception fathers
  - Occupational
    - Positive results
- During pregnancy for mothers
  - Occupational
    - NS
  - Residential
    - NS
- Child exposure post-natally
    - Positive results

New results (ALL-parental occupation)

Infante-Rivard et al.

- Using the so-called expert method (Gérin et al., 1985; Siemiaticky et al. 1987)
  - chemists code the exposure based on classification of job, industry, description of work practices and environment, etc. and using general and specific questionnaires
- Methods described for maternal occupational exposure to solvents
  - Infante-Rivard et al. Environ Health Perspect 2005; 113:787-92

New results (ALL-parental occupation)

Infante-Rivard et al.

	Cases N (%)	Controls N (%)	OR (CIs)	Ratio of discordant pairs	Adjusted OR (CIs)*
Paternal exposure during the 3 months before to pregnancy					
Biocides	77 (9.8)	73 (9.3)	1.06 (0.76-1.49)	68:64	1.07 (0.76-1.49)
Fertilizers	28 (3.6)	12 (1.5)	2.33 (1.19-4.59)	28:12	2.44 (1.23-4.84)
Pesticides	88 (11.2)	62 (7.9)	1.51 (1.06-2.15)	77:51	1.48 (1.05-2.09)
Fungicides	16 (2.0)	3 (0.4)	5.33 (1.55-18.30)	16:3	5.45 (1.58-18.78)
Insecticides	64 (8.1)	50 (6.4)	1.33 (0.89-1.97)	57:43	1.33 (0.90-1.95)
Herbicides	20 (2.5)	11 (1.4)	1.82 (0.87-3.79)	20:11	1.87 (0.89-3.93)

* adjusted for age and sex of the children

New results (ALL-parental occupation)

Infante-Rivard et al.

	Cases N (%)	Controls N (%)	OR (CIs)	Ratio of discordant pairs	Adjusted OR (CIs)*
Materna; exposure during pregnancy					
Biocides	95 (12.0)	99 (12.5)	0.95 (0.70-1.30)	79:83	0.95 (0.70-1.28)
Fertilizers	7 (0.9)	6 (0.9)	1.20 (0.37-3.93)	6:5	1.15 (0.38-3.44)
Pesticides	34 (4.3)	35 (4.4)	0.97 (0.59-1.60)	30:31	0.97 (0.60-1.57)
Fungicides	4 (0.5)	4 (0.5)	1.00 (0.25-4.00)	4:4	0.95 (0.24-3.85)
Insecticides	27 (3.4)	23 (2.9)	1.19 (0.67-2.13)	25:21	1.20 (0.68-2.11)
Herbicides	5 (0.6)	8 (1.0)	0.50 (0.13-2.00)	3:6	0.63 (0.20-1.92)

* adjusted for age and sex of the children

Genetic variants as modifiers of the effect of pesticides on chidlhood cancer

- So far, very limited investigation
- There are reasonable biological arguments to study modifying effects of gene variants on pesticides, and plausible pathways (e.g., metabolizing genes and others) can be selected
- However, overall, results do not meet high enough standards

Genetic variants as modifiers of the effect of pesticides on chidlhood cancer

- Sample size issue:
  - Numbers in GxE studies and numbers in GWAS studies so far (even with no E measures reported) are not consistent with a proper investigation of GxE in childhood cancer
- Others major issues are related to quality assurance and quality control criteria which have not been stringent enough to give strong and credible results

Genetic variants as modifiers of the effect of pesticides on chidlhood cancer

- There are two huge challenges in the equation:
  - Measurement of *environmental exposure*
    - QA and QC criteria are not established
    - At this stage, we are lacking innovative, feasible, and more accurate measures applicable in population-based studies
    - The weakness of our methods seem to lead to (and possibly justify) endless repetitions of the same studies
    - Nevertheless, the interpretation of the collected E data is simple and even binary classifications carry information
    - Similar positive results over many studies (however limited) are indicative of causality
  - QA and QC for the *genetic component* of the equation

QA and QC for genetic variants

- Quality assurance: good design, DNA, DNA extraction procedures, call rates (signal intensity plots or clusters)
- Quality Control (filter individuals and SNPs)
  - Individual-specific QC
    - Missingness (informative)
    - Gender check
    - Duplicates and cryptic relatedness (using LD pruned dataset)
    - Population outliers (admixture; PCA)
    - Heterozygosity (high=sample contamination and low= inbreeding) (departure from HWE)
  - SNP-specific QC
    - Missingness (call rate=prop non-missing SNP/n individuals)
    - Minor Allele Frequency variants
    - HWE (extreme departure likely due to calling errors)
- Multiple testing adjustment

Plausibility of overall results from environmental epi studies

- Results are consistent, which is indicative of causality
- More specifically, there is consistency over 3 time windows of possibly greater biological relevance:
  - Occupational exposure of fathers during preconception periods
  - Occupational and residential exposures of mothers during pregnancy
  - Direct (residential) exposure post-natally

Plausibility of overall results from environmental epi studies

- There is still a chance that consistent results could be wrong
- Thererefore two important points are:
  - Is there *biological plausibility* to the rather consistent link observed in epi studies of between pesticides and childhood cancer
  - Why are the results from *regulatory agencies* not consistent with the epidemiological results?

Plausibility of overall results from environmental epi studies

- Biological plausibility
  - Little discussion needed for maternally-mediated effects (pregnancy) and for direct effect on the child
  - Among the more consistent results are the *paternal preconception exposures* which have not been given much credibility fot lack of plausible mechanisms

Biological plausibility
Cell Metabolism Feb 2011
paternal preconception exposures

Cell Metabolism
Previews

You Are What Your Dad Ate

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DOI 10.1016/j.cmet.2011.01.011

Maternal nutrition and metabolism are critical determinants of adult offspring health. Recent reports describe adverse offspring outcomes associated with the *father's* diet, indicating nongenetic inheritance of paternal experience. Determining underlying mechanisms may require reconsideration of our understanding of the heritability of epigenetic states.

Biological plausibility
Nature Rev Genet Feb 2011
paternal preconception exposures

EPIGENETICS

Dad's diet lives on

Two recent studies in rodents show that unhealthy paternal diets can reprogramme gene expression in offspring, implicating epigenetics in these transgenerational effects.

Although there is increasing evidence for effects of parental environment in offspring, these studies add to just a handful of cases in which the molecular basis has been at least partly elucidated. Clearly, the role of epigenetics in such transgenerational effects will be an important focus of future studies.

ORIGINAL RESEARCH PAPERS Ng, S.-F. *et al.* Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* **467**, 963–966 (2010) | Carone, B. R. *et al.* Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* **143**, 1084–1096 (2010)

Biologogical plausibility
Nature and *Cell* papers

Ng and colleagues fed male rats a high-fat diet and looked for effects in their adult female offspring, which were fed a normal diet. These daughters had normal body fat but showed signs of pancreatic β-cell impairment and altered expression (as compared to controls) of 642 genes that are involved in pathways related to insulin regulation and glucose metabolism. The gene with the greatest alteration in expression was interleukin-13 receptor-α2 (*Il13ra2*), which is implicated in regulating pancreatic cell function. Interestingly, DNA methylation at a cytosine residue close to the *Il13ra2* transcriptional start site was reduced in these females.

Carone and colleagues looked at the effect of a paternal low-protein diet in mice. Offspring of both sexes showed altered gene expression compared to controls, including genes involved in fat and cholesterol biosynthesis, consistent with physiological differences in these mice. Modest changes in DNA methylation were seen at many sites, including a reproducible change close to the *Ppara* gene, which encodes peroxisome proliferator-activated receptor-α, a regulator of lipid metabolism.

Biological plausbility

Paternal Environmental Exposures and Gene Expression during Spermatogenesis: Research Review to Research Framework

The primary objective is to review Dioxin toxicity, the potential impact on spermatogenesis, what is known and unknown about paternal exposures, and the potential mechanisms whereby paternal preconception exposures result in neural tube defects (NTD). The secondary goal is to suggest a versatile research framework utilizing gene expression microarray to evaluate the impact of acute, intermittent, and chronic paternal exposures to environmental agents on gene expression during the stages of spermatogenesis. There are multiple barriers to establishing a paradigm whereby paternal environmental exposures result in adverse birth outcomes. Microarray expression studies are unique in their ability to detect transcription dysregulation, thereby facilitating the identification of molecular and developmental pathways through hierarchical and pathway analysis. To date there are no studies of gene expression during spermatogenesis following exposure to environmental agents. **Birth Defects Research (Part C) 84:155–163, 2008. Published 2008 Wiley-Liss, Inc.**†

Biological plausibility

Microarray studies Vinuela et al. *Plos One* Aug 2010

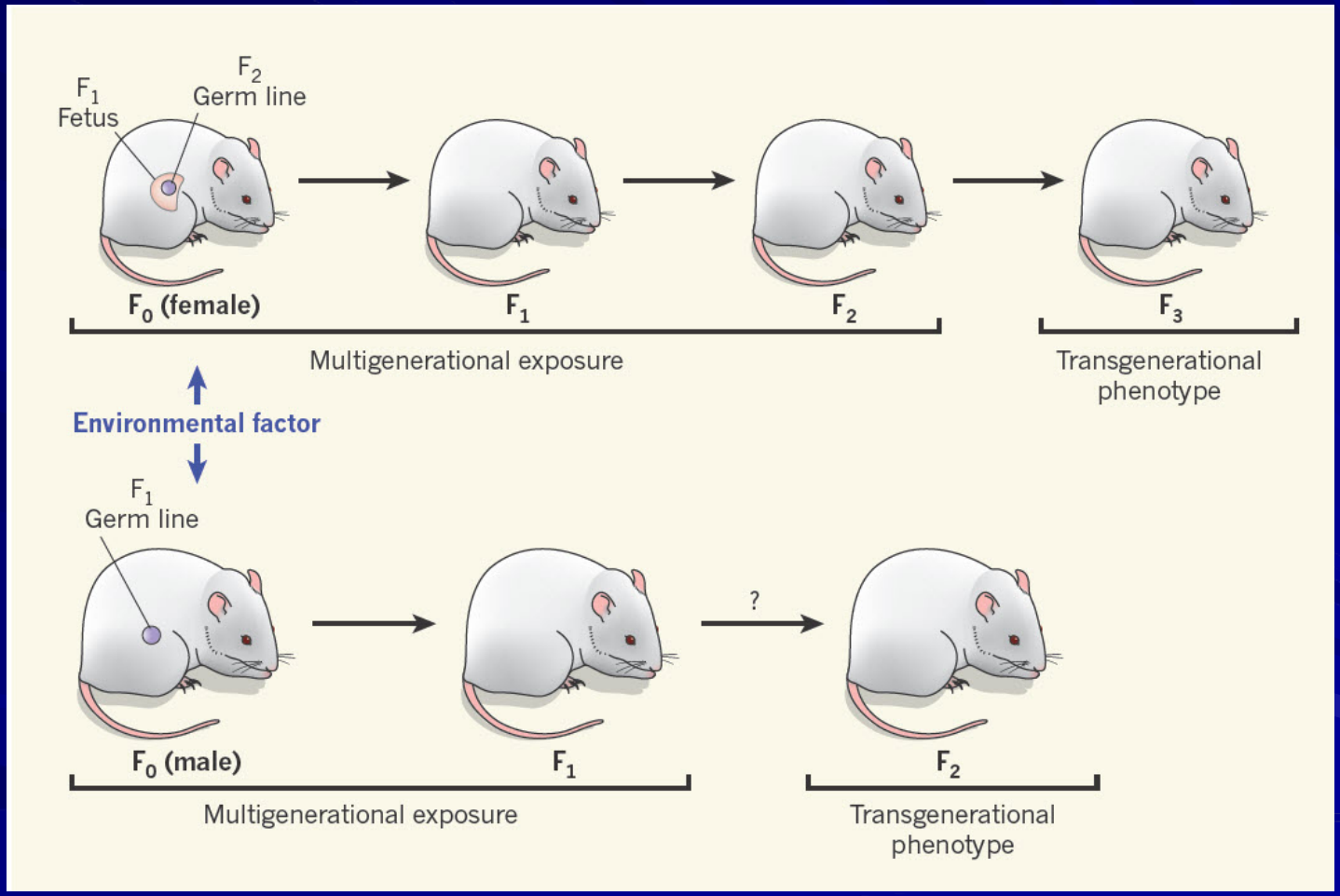
- Investigators performed a microarray study in C. elegans exposed for 72 hrs to two widely used Ops, chlorpyrifos and diazinon, and a low dose mixture of these two compounds.
- They observed transcriptional responses related to detoxification, stress, innate immunity, and transport and metabolism of lipids in all exposures. For both compounds as well as in the mixture, these processes were regulated by different gene transcripts.
- These results illustrate intense, and unexpected crosstalk between gene pathways in response to chlorpyrifos and diazinon in C. elegans.

Biological plausibility

New biological avenues for maternal effects *Frontiers in Genet* Apr 2012

- Many relatively common environmental exposures,such as cigarette smoking, alcohol consumption,and drug use, may lead to aberrant expression and function of non-coding RNA(ncRNA) (in particular microRNA (miRNA), piRNA, and long ncRNA), which are important post-transcriptional regulators of gene expression.
- During pregnancy cigarette smoke might dysregulate miRNA expression in different placental cell types
- These alterations may have consequences throughout the life course
- And consequences across generations, but this has not been shown yet

Biological plausibility
Transgenerational effects (*Nature* Oct 2010)



Plausibility of epi studies vs regulatory agency decisions

- Pesticides are approved for use before being put on the market (US, Canada, Europe, etc.)
- Therefore the pesticides we studied are considered safe
- Le Monde (April 3, 2012)
  - Pesticides: Les autorisations “laxistes” de l’Europe
    - Une dizaine de substances suspectes reviennent sur le marché
    - “Homologation au rabais” (watered-down)
    - Manque de données

Plausibility of epi studies vs regulatory agency decisions

- Limits of toxicological tools currently used: very high doses used, extrapolation from animal studies, use of adolescent animals (no direct studies in utero, on children, over lifetime)
- Agencies approving the marketing of pesticides (in Canada and the US) use approaches that are 50 years old
- Animal testing is done by industry or contracted labs, and their data are reviewed by the agencies (all in high secrecy based on proprietary concerns)

Plausibility of epi studies vs regulatory agency decisions

Advancing Regulatory Science. *Science* 2010;331:(6020)987
Margaret Hamburg (Commissioner, FDA)

- *"Today, we are neither effectively translating scientific discoveries into therapies nor fully applying knowledge to ensure the safety of food and medical products. We must bring 21st century approaches to 21st century products and problems..."*

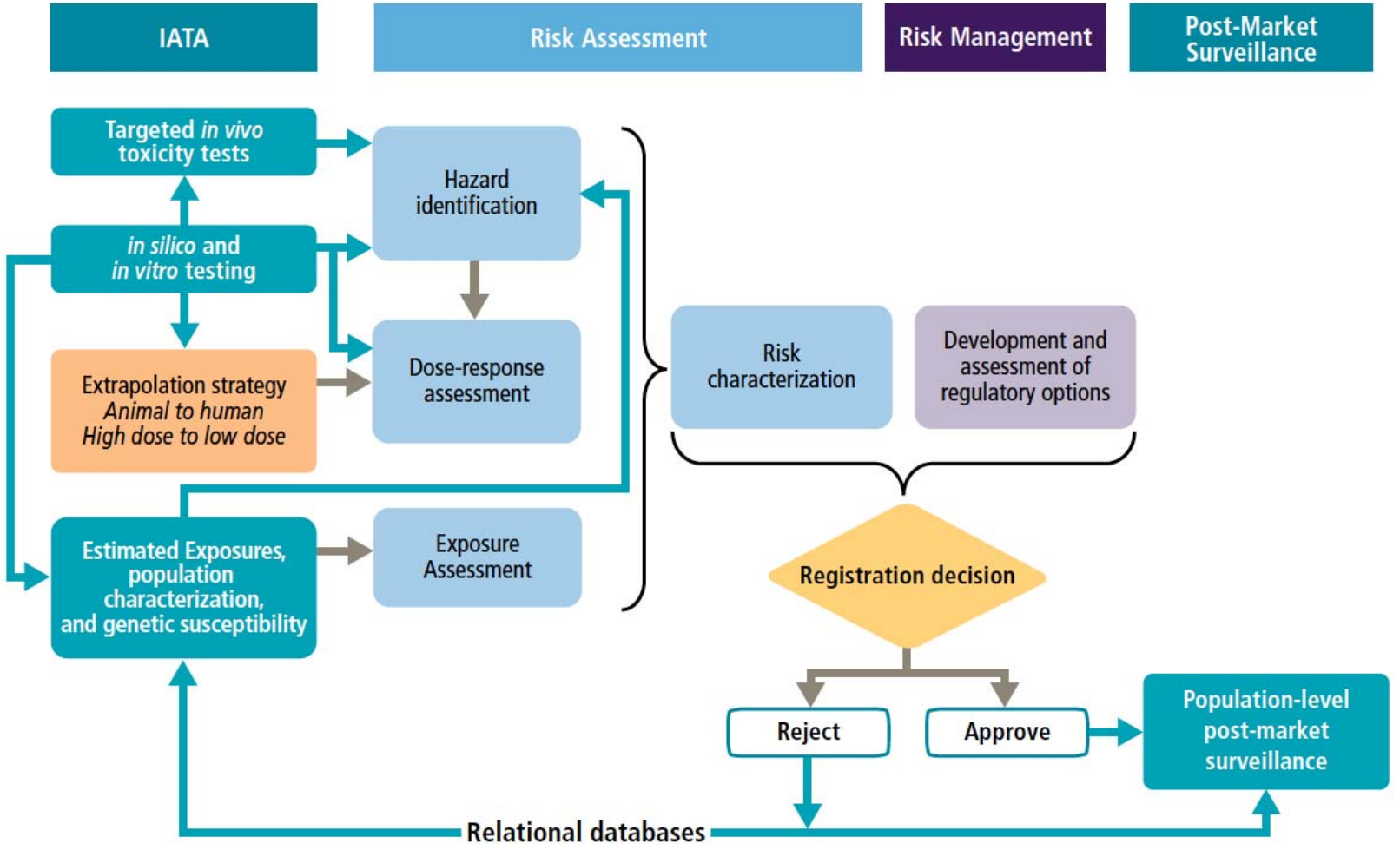
- *"Most of the toxicology tools used for regulatory assessment rely on high-dose animal studies and default extrapolation procedures and have remained relatively unchanged for decades, despite the scientific revolutions of the past half-century.*
- *We need better predictive models to identify concerns earlier in the product development process to reduce time and costs. We also need to modernize the tools used to assess emerging concerns about potential risks from food and other product exposures…"*

Plausibility of epi studies vs regulatory agency decisions

Alternative approaches to tox testing for regulatory agencies

- Council of Canadian Academies. Expert Panel.
  - Integrating emerging technologies into chemical safety assessment (2012)
  - IATA (integrated approach to testing and assessment of chemicals)

Chapter 6: THE ROAD AHEAD



Plausibility of epi studies vs regulatory agency decisions

Chemical Toxicity Screening (*JAMA* Jan 2012)

- More than 10 000 chemicals will be screened for potential toxic effects on human health, as part of joint effort by the NIH, the EPA, and the US FDA.
- The Tox21 project aims to use emerging technologies to better assess whether currently used compounds pose risks and to help drug developers identify potential toxicities earlier in the drug development process.
- A robotic screening system will be used to determine whether selected compounds or compound mixes can disrupt biological human processes and lead to adverse effects

Plausibility: alternative explanations for epi results

- Let's assume three arguments in support of an association pesticides-childhood cancer:
  - Consistency of results
  - Biological plausibility of results
    - Newly uncovered mechanisms (non-coding RNAs)
    - Apparently implausible results (paternal preconception) provided with newly uncovered plausible mechanisms (altered gene expression and DNA methylation)
  - Discrepancies between regulatory agency decisions and epi study results may have many resonable explanations

Plausibility: alternative explanations for epi results

- What about QA (study design) for epi?
  - There is certainly large *measurement* error for exposure to pesticides, but no data that I know of document differential misclassification (here I am inspired by parental smoking data)
  - A more likely and difficult problem is *selection* bias
    - Very difficult to determine from published reports
    - Would most likely arise from low participation rates in eligible controls resulting in actual study controls not being representative of the base (more educated and less exposed than the base resulting in overestimation of OR)

Plausibility: alternative explanations for epi results

- Residential exposure studies reviewed for possibility of selection bias (*JESEE* 2010)
- Main sources of potential bias were:
  - a non-concurrent selection of controls with respect to cases
  - the use of control diagnoses possibly caused by pesticide exposure in hospital-based studies
  - non-participation of selected eligible subjects.
- A sensitivity analysis varied prevalence of E in eligible Ca & Co who were selected
  - we concluded that non-participation alone could not explain the reported positive associations.

Conclusions

- Despite study limitations (imperfect exposure measures, need for a genetic component)
- Despite discrepancies in our results with the decisions of regulatory agencies
- The data on pesticides-childhood cancer (leukemia in particular) are consistent, biologically plausible in all time windows, and glaring biases not documented
- But, could we still be missing something that would invalidate our results?