Health Canada / Santé Canada
Pest Management Regulatory Agency / Agence de réglementation de la lutte antiparasitaire

Date Received - Date de réception :

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Notice of Objection to a Registration Decision under Subsection 35(1) of the *Pest Control Products Act*

Avis d'opposition à une décision d'homologation en vertu du paragraphe 35(1) de la *Loi sur les produits antiparasitaires*

1. Objector information - Information sur l'opposant

Name - Nom / Corporation - société / Organization - organisation: Meg Sears.

Postal Address - Adresse postale: RR 1, Box 9012 Dunrobin, Ont.

City/Town - Ville	Province/State - Province/État	Country - Pays	Postal Code/ZIP - Code postal/Zip
Dunrobin.	Ont.	Canada	K0A1T0.

Phone - Téléphone	Fax - Télécopieur	E-mail - Adresse électronique
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2. Product information - Information sur le produit

Name of active ingredient to which the decision relates:
Nom de la matière active à laquelle la décision se rapporte : 2,4 dichlorophenoxyacetic acid (2,4-D).

Name of end-use product to which the decision relates:
Nom de la préparation commerciale à laquelle la décision se rapporte : all products and mixtures containing 2,4-D acid, salts & esters

3. Registration decision to which the objection relates - Décision d'homologation pour laquelle vous déposez un avis d'opposition

Decisions on application - Décision concernant la demande

- ☐ Granting registration - Homologation accordée
- ☐ Denying registration - Homologation rejetée
- ☐ Granting an amendment of a registration - Modification à l'homologation accordée
- ☐ Denying an amendment of a registration - Modification à l'homologation rejetée

Decisions on re-evaluation or special review - Décision concernant la réévaluation ou l'examen spécial

- ☒ Confirming registration - Homologation confirmée
- ☐ Cancelling registration - Homologation annulée
- ☐ Amending registration - Modification à une homologation

4. Date the decision statement was made public: Date de la publication de l'énoncé de décision : May 16, 2008 (deadline was extended)

5. Area of scientific evaluation to which the objection relates - Volet de l'évaluation scientifique touché par l'avis d'opposition

- ☒ Health risk assessment (toxicology, food residue, occupational exposure) & epidemiology
  Évaluation des risques pour la santé (toxicologie, résidus dans les aliments, exposition professionnelle)
- ☐ Environmental risk assessment (environmental fate, environmental toxicology) -
  Évaluation des risques pour l'environnement (devenir dans l'environnement, écotoxicologie)
- ☐ Value and efficacy assessments (crop tolerance, value) -
  Évaluation de la valeur et de l'efficacité (tolérance des cultures, valeur)

6. Scientific basis for the objection / Fondement scientifique de l'opposition — *Attachment included: / Pièce jointe incluse :* ☒ Yes ☐ No / ☐ Oui ☐ Non

objection document is being emailed to infoserve.

pmra_infoserve@hc-sc.gc.ca

M. E. Sears	M. E. Sears
7. Signature of objector or representative - Signature de l'opposant ou de son représentant	**Printed Name - Nom en lettres moulées**

Objectors who submit confidential information (i.e., confidential business information, confidential test data) are responsible for identifying this information which is part of their submission.

Information required to process the notice of objection may include some personal information as defined in the *Privacy Act*. In accordance with that Act, such personal information may be made public as authorized by the *Pest Control Products Act* and its regulations. Under the *Privacy Act*, individuals have the right to look at their personal information. For more information on how PMRA manages personal information, contact the PMRA Information Services at 1-800-267-6315 within Canada and 1-613-736-3799 outside of Canada or via e-mail at pmra_infoserv@hc-sc.gc.ca.

Les opposants qui soumettent des renseignements confidentiels (c.-à-d. des renseignements commerciaux confidentiels, des données d'essai confidentielles) sont responsables de les désigner comme tels dans leur envoi.

L'information requise pour traiter cet avis d'opposition peut comprendre certains renseignements personnels tels que définis dans la *Loi sur la protection des renseignements personnels*. Conformément à cette Loi, ces renseignements peuvent être rendus publics, ce qui est permis par la *Loi sur les produits antiparasitaires* et son Règlement. En vertu de la *Loi sur la protection des renseignements personnels*, tous les individus ont le droit de consulter leurs renseignements personnels. On peut obtenir des précisions sur la gestion des renseignements personnels auprès de l'Agence de réglementation de la lutte antiparasitaire (ARLA) en communiquant avec le Service de renseignements au 1-800-267-6315 au Canada, ou au 1-613-736-3799 de l'extérieur du Canada, ou par courrier électronique à pmra_infoserv@hc-sc.gc.ca.

Notice of Objection to a Registration Decision under Subsection 35(1) of the *Pest Control Products Act*

Regarding registration of 2,4-dichlorophenoxy acetic acid (2,4,-D)
(RVD2008-11, May16, 2008)

Meg Sears PhD **August 15, 2008**
RR1, Box 9012, Dunrobin, Ontario, K0A 1T0
Telephone: 613 832-2806 (call ahead for FAX) **Email: megsears@ncf.ca**

I am objecting to the Pest Management Regulatory (PMRA) decision to re-register for the sale and use in Canada products containing 2,4-D, including the acid, salts and esters.

I request that:

- the use of phenoxy herbicides (including 2,4-D) be discontinued for turf and to be curtailed for all but the most urgent uses (should any exist);
- all dioxin contaminants be more strictly regulated, and be monitored in herbicide products, the environment, foods and people; and
- pesticides be assessed using modern medical scientific methodologies and standards, including consideration of epidemiology, the diversity of people's physiology, and other forms of scientific evidence.

Background

The Pest Control Products Act (PCPA 2002) stipulates that pesticides may be registered if there is "reasonable certainty that ***no*** harm to human health, future generations or the environment will result from exposure to or use of the product …" (emphasis added). I believe that this represents a much higher regulatory bar than is evident in the PMRA documents and decision regarding 2,4-D.

Four documents have been published in recent years by the PMRA regarding 2,4-D:

- Re-evaluation of the Lawn and Turf Uses of (2,4-Dichlorophenoxy)acetic Acid [2,4-D]. Proposed Acceptability for Continuing Registration (PACR2005-01), 21 February 2005
- Lawn and Turf Uses of (2,4-Dichlorophenoxy)acetic Acid [2,4-D]. Interim Measures. Re-evaluation Note (REV2006-11), 16 August 2006.
- Re-evaluation of the Agricultural, Forestry, Aquatic and Industrial Site Uses of (2,4-Dichlorophenoxy)acetic Acid [2,4-D]. Proposed Acceptability for Continuing Registration (PACR2007-06), 19 June 2007.
- Re-evaluation Decision (2,4-Dichlorophenoxy)acetic Acid [2,4-D] (RVD2008-11), 16 May 2008.

Concerns regarding pesticide regulation in Canada and the phenoxy herbicide 2,4-D in particular, following comments on PACR2005-01, were addressed in a 2006 peer-reviewed article published in Paediatrics and Child Health (the journal of the Canadian Paediatrics Society), provided as Appendix A.[1]

Subsequently, I have submitted comments on PMRA documents, corresponded with the PMRA, met with staff in August of 2007, and visited the Reading Room twice in preparation of this objection.

In this objection, scientific deficiencies in both data and methodology for synthesizing information are discussed. As well, suggestions are made to improve synthesis of information about toxic chemicals for the purposes of regulation.

Summary

The following form the basis of this objection:

1. Industry-supplied confidential animal toxicology reports under-estimate toxic effects. For example, symptoms such as excessive salivation or red material around eyes are not considered to be toxic effects. They should be - such symptoms are reported to occur in the majority of animals at doses well below the "No Observable Adverse Effect Level" (NOAEL). As well, evidence of toxicity (e.g. average body weight is half that of the control group) may not be statistically significant because either the experiments were poorly conducted so the scatter in the data is excessive, or too few animals were used. Only extreme toxicities (typically death) emerge as significant. In some key reports, toxic effects actually occurred at all doses. A particularly important point is that the chronic exposure NOAEL was inappropriately increased from 1 mg/kg bw/day (milligram per kilogram of body weight per day) to 5 mg/kg bw/day, after examination of new sections of old samples of rat kidneys. In addition, I bring to the attention of the PMRA a recent study in rats showing that exposure to as little as 10 mg/kg bw/day 2,4-D during the first post partum days produced changes in maternal behavior, serum prolactin and monoamine levels in the AcN of treated dams.[2] This is in contrast to the 12.5 or 25 mg/kg bw/day short term NOAELs incorporated in the 2,4-D re-assessment. Correction of these short term and chronic values should lead to recalculation of the acceptable human exposure, including an additional 10-fold factor for extrapolation from a "Lowest Observable Adverse Effect Level" (LOAEL) to a NOAEL. Proper incorporation of this new LOAEL into the assessment may preclude some or all uses of 2,4-D.

2. There is no dispute that dioxins contaminate 2,4-D. There is an economic incentive to produce herbicides with higher levels of contamination because dioxins are produced in greater quantity at higher reactor temperatures; the same conditions under which production is more rapid and conversion more complete. Outstanding questions are the extent of contamination of products used in Canada, and the potential health impacts of dioxins with fewer than 4 chlorine atoms:
    a. measures proposed by the PMRA in the RVD2008-11 to obtain contaminant information from the applicants will not be reliably informative. Manufacturers monitor reactor temperature and dioxin contamination, so samples and data are readily selected by the applicants and may not be representative of products in use in Canada. The PMRA should review all production contaminant data from companies over the past 5 years, and conduct its own tests of off-the-shelf phenoxy herbicides.
    b. in published tests of immune suppression, the principle dioxin contaminant of 2,4-D was of potency similar to the most toxic dioxin. This is also the interpretation of the Agency for Toxic Substances and Disease Registry in the United States. The PMRA misrepresented the toxicity of 2,7-dichlorodibenzo-para-dioxin in the RVD2008-11.
    c. Research is urgently needed on the health impacts of lower chlorinated dioxins, as well as their prevalence in the Canadian population and environment, as well as foods.

3. Human epidemiology is not only afforded little weight in pesticide assessment, there is a major error in interpretation of a landmark study of non-Hodgkin lymphoma (nHL) in the RVD2008-11. This is a very serious, fundamental matter, calling into question competence in epidemiology and interpretation of statistical information. The 2006 study by Chiu *et al*.,[3] in concert with numerous other studies, clearly links phenoxy herbicides with the most intractable, most rapidly increasing type of nHL. The epidemiology literature is not comprehensively represented in the PMRA bibliography, and given the findings of the studies listed in successive publications there is good reason to believe that only studies that were pointed out by others are actually included in the list (pesticide proponents provided the literature referenced in the

PACR2005-01 and studies pointed out by others were mentioned in successive documents). Similarly, other types of studies in the public, peer-reviewed literature were not systematically assembled or reviewed. Within the past few years, numerous studies (the preponderance of those reported) had significant findings of harms from phenoxy herbicides and 2,4-D in particular. Significant findings have been found for nHL, as well as for leukemia, breast cancer and brain tumours in people. Epidemiological studies should be used in pesticide assessment, or as they become available in subsequent adaptive management of the hypothesis that their use will not cause harm.

4. The epidemiological, biochemical and toxicological literature is not systematically searched and synthesized to put together the scientific "puzzle-pieces." There is no systematic mechanism at the PMRA to do this. However, members of Canada's medical community who have done so have repeatedly expressed concerns that 2,4-D is persuasively linked to cancers, and reproductive, developmental and neurological problems. I was informed that when an epidemiological or other study comes to staff's attention, if the single study, in and of itself, is sufficient to overturn the decision then action would be taken. This is a very unlikely scenario.

5. There is a disconnect between the animal toxicology studies and human observational studies, in that all animal studies are conducted at doses that are orders of magnitude higher than human exposures. PMRA staff repeat the simplistic, 16th century mantra favoured by the pesticide industry, that "the dose makes the poison." However, today we know that non-linear dose responses are common, and even exploited with some medications. Non-linear responses are common with chemicals that have hormonal or immune system effects (effects that are reported with 2,4-D), and in my brief survey of confidential test data non-linear responses were reported in animal toxicity studies (these were always dismissed as scatter in the data). The rationalization for using very high test doses is to elicit responses in greater numbers of animals, and even that lag times for delayed effects may be shortened at higher doses. However, it must be recognized that high-dose testing is an artefact of the assessment *system*. No tests will be conducted by the industry at doses that could not withstand application of the extrapolation factors, and remain above the human exposure estimates. Reverse engineering of experimental design in this context means that no testing will ever replicate levels of human exposure, and thus low dose effects will never be observed. This is why non-industry funded research is essential. The government should support university and governmental research into toxic effects of pesticides and other prevalent, potentially toxic chemicals.

6. Developmental and neurological toxicities have long been a concern, and the PMRA in its decision is calling for "confirmatory" data from a new study. The studies which are to be confirmed are poorly conducted and out of date (reports are from around 1990), and are not a sound basis for the decision to continue registration of 2,4-D. The design for the new study is presently being discussed. I am not an expert in assessing neurotoxicity endpoints in animals but am unaware of validation with endpoints such as autism or attention deficit. However, in addition to the measures mentioned in the recent work referenced in point 1 above, the following basic study-design recommendations are made to improve the proposed research in accordance with modern standards:
   a. extend the proposed study to 3 generations in total (two births), since previous research appeared to demonstrate effects at that stage;
   b. include extra doses that are environmentally relevant;
   c. use random methods to sample and pool production concentrates for testing, rather than using a highly purified form of 2,4-D that would be devoid of toxic contaminants;
   d. blind researchers as much as possible as to treatment groups;

e. power the experiment to detect differences in readily measured parameters such as feed consumption and body weight;
f. analyse all feeds and used bedding to ensure that they are not inappropriately contaminated with 2,4-D, breakdown products or contaminants, or with other materials that may affect the outcome of the experiments (recent research has shown that animal feeds may skew results of toxicology experiments when endocrine effects are under investigation); and
g. analyse data and experimental standards independently.

7. Clearly the 2,4-D information within the Agency is in disarray. Visits to the Reading Room were very informative. PMRA staff members were uniformly welcoming and appeared to be very cooperative. However, staff has yet to provide me with a single, clear bibliography of reports considered during the reassessment. Most recently, two bibliographies were provided that between them purportedly include all references. One list includes five partial references (journal page numbers only – no authors or titles) that were pointed out to the PMRA following publication of the decision. In general references have no consistent format and many are incomplete; indeed, many entries are unidentifiable due to missing information. During the second day in the Reading Room, when the studies were finally on the computer, some key, large electronic documents were not searchable. Thus searches for keywords such as "dioxin" or "child" were not comprehensive.

8. Making the PMRA Data Evaluation Reports (DERs) or the equivalent available was a key recommendation of the Reading Room Pilot Project, but this has not yet been acted upon. They should be publicly available as they would allow one to discern the logic and decision-points of the assessment process.

9. Research involving mixtures of pesticides is allotted minimal if any relevance, despite the fact that 2,4-D is almost always used in mixtures (a single one-pesticide product is registered), and that all products for turf (where the largest number of people are exposed) are mixtures. The PCPA requires cumulative assessment of pesticides with common mechanisms of toxicity, but phenoxy herbicides were not assessed in this manner. Pesticide assessment should take into account the way the material is used, not only in terms of application methods, but also other pesticides plus ingredients that may affect penetration/absorption, metabolism and toxicity.

In coming to the present decision to continue registration of 2,4-D, the PMRA omitted consideration of relevant publicly available information, and apparently accepted poorly executed and reported industry studies. 2,4-D and doubtless other phenoxy herbicides should be discontinued for all uses but the most urgent ones (should any exist). The PMRA should conduct and/or support independent investigation of toxic contaminants.

When registering a pesticide, the Pest Control Products Act 2002 stipulates that there must be "reasonable certainty that ***no*** harm to human health, future generations or the environment will result from exposure to or use of the product …" (emphasis added). This is a high regulatory standard that has not been met in the present case. This statement must be treated as a scientific hypothesis, to be investigated and either validated or disproved. The scientific plan for testing of the no-harm hypothesis should be laid out in the decision. Until the hypotheses generated by toxicological risk assessment are validated by monitoring of human and environmental contamination, epidemiological research and other basic science studies, Canada's regulatory system is not truly "science-based."

The PMRA should reconsider, once again, its communications policy. It was wise and prudent for the PMRA to remove references to "safe" from its website and to use more circumspect language. The recent return to referencing "safe" encourages complacency, and facilitates pesticide applicators circumventing the PCPA provisions not to advertise "safety."

Information considered for the 2008 2,4-D decision

It is unclear what information actually was considered during the reassessment. As of the time of this submission I have not received a single, clear, complete list of reports actually considered during the re-assessment.

The RVD2008-11 states repeatedly that a complete bibliography of all information considered for the decision is available upon request. My first request of June 5th, 2008 was acknowledged June 6th, but the bibliography did not come. Despite numerous reminders, on July 7th, 2008 I had still received no meaningful response so I requested to visit the Reading Room. The recommended protocol for utilizing the Reading Room is to obtain the bibliography and to identify the information one would like to view. However, with no bibliography in hand and the July 15th objection deadline approaching I made an appointment to visit the Reading Room on July 10th, 2008 (the deadline was extended for a month following this debacle). July 9th I received by email a bibliography in Word format containing fewer than 300 entries. I asked for confirmation that this was indeed the entire bibliography (it appeared skimpy), but this was not forthcoming. During my July 10th visit to the Reading Room I was informed that the bibliographic list was actually much longer. The re-evaluation had been ongoing for such a long time that the bibliography was spread amongst several databases and had never been pulled together.

During the July 10th visit PMRA staff attempted to obtain and upload the missing information onto the Reading Room computer, but this was still not accomplished as of 6pm. Following this rather fruitless first visit, I received two more documents (an excel file and a pdf) with lists of references. Between the two, the references were supposed to be complete, albeit with overlaps. The excel file included a set of five references in brief form (journal, year, volume and page numbers only – no authors or titles) just as they were mentioned in a letter following publication of the decision. Some of these were not available at the time of publication of the decision, and presumably had the reports actually been reviewed the references would have been completed. Thus, this reference list was probably bloated with material that had not been considered for the decision. Also, many references in the list were incomplete or truncated, to the point that it would be impossible to identify a specific article. There was no consistent format for the references (e.g. number of authors cited, order of information). What sorting there was, was wrong. For example, "Unpublished" information included some peer-reviewed articles that are available free online, some that are published in publicly accessible journals with restricted access, and even a Health Canada public information sheet that is available free upon request and online.

With the reports in disarray, and in the absence of systematic searching for and evaluation of scientific reports in the public domain, I seriously doubt that systematic consideration could have taken place. Even small, discrete questions of toxicology, for which information was assembled by the proponents, would not have been supplemented with other information. Global synthesis of information is not possible in this situation. Moreover, PMRA staff indicated that when an epidemiological study comes to their attention, that they examine it. If the single study, in and of itself, by itself, is sufficient to overturn the decision then action would be taken. This is a very unlikely scenario. The epidemiological, biochemical and toxicological literature is not systematically searched and synthesized to put together the scientific "puzzle-pieces." It should be.

Information upon which the 2008 2,4-D decision was based

In preparation for my second visit to the Reading Room I requested identification of the reports on with the NOAELs were based. The body of this email is reproduced in Appendix B. I had insufficient

time in the Reading Room to review all the studies in detail (each document is typically over 100 pages).

It is puzzling that the same set of reports (PMRA# 1291538 (BEE); #1292201(IPA); #1311889(DEA); #1370428 (acid); #1373794 (EHE); #1379309 (DMA); #1417864 (TIPA)) were key to setting both a Female Acute Dietary NOAEL of 25 mg/kg bw/d and an aggregate (1-7 day) general population NOAEL of 12.5 mg/kg bw/day. The lowest dose used in some studies was 25 mg/kg bw/day, while 12.5 mg/kg bw/day was used in others. Unless other studies that I did not examine utilized still lower doses at which no effects were observed, these values are actually LOAELs, not NOAELs. In the RVD2008-11 it was stated that no LOAELs were used in the final decision. This is important because use of a LOAEL should entail an additional 10-fold extrapolation factor.

The studies listed above were conducted in 1989-1990, but were not supplied to the PMRA until after the PACR2005-01. One has to wonder why, in the interest of full disclosure, they were not provided previously. Generally these studies using small groups of animals did not entail extensive biochemical or haematological monitoring. Importantly, salivation and reddish material about the eyes were commonly noted at lower doses, but the authors did not consider these to be evidence of toxicity. However, salivation is commonly known to be evidence of adrenergic effects, as reported for instance by Ohshika et al in 1978.[4] Nor were the studies powered to detect anything other than the most overt problems, so death is commonly the reported outcome at the LOAEL. The scatter of the data was so great that in one study even when the average maternal weight at necropsy was half the weight of the control group it was not statistically significant. In this context "no evidence of a toxic effect" is apparently considered to be "evidence of no toxic effect," which is clearly preposterous.

Examining new sections of old rat kidneys led to a five-times increase in allowable exposure

In the PACR2005-01, the chronic exposure NOAEL was 1 mg/kg bw/day, based upon chronic progressive nephropathy at higher doses in long-term studies in rats. The present chronic exposure NOAEL is 5 mg/kg bw/day. The change occurred because the Industry Task Force II for 2,4-D Research hired an expert to prepare new slides from preserved kidney specimens (the previous slides were said to have been lost), and to re-examine this question. The new analysis, using the decades-old samples, concluded that there was no evidence of toxicity in kidneys of animals dosed with 1 and 5 mg/kg bw/day, so the NOAEL was revised upwards by a factor of 5. In other words, based upon this re-examination, Canadians may be exposed to five times as much 2,4-D.

This *post-hoc* overturning of previous conclusions based upon aged, possibly sub-optimal samples should not have been entertained by the PMRA. It is not only overtly suspect that previous scientific findings would be over-turned by a second opinion examination of "left-over" samples, there are serious scientific issues.

With respect to such reconsideration of pathology, Dr. Robert Fraser MD, FRCPC, Head, Anatomical Pathology at IWK, and Associate Professor of Pathology at Dalhousie University in Halifax, kindly provided the following comments:

- If in fact the slides were lost, additional slides can be cut from the paraffin embedded tissue blocks if they can be reliably located and retrieved. Hopefully fresh frozen tissue was not postfixed and used, as this would introduce artifacts caused by the freezing and thawing of the tissue. It would be interesting to know how many samples from each kidney were submitted as well as the number of slides reviewed from each of the tissue blocks (i.e. the number of stained sections). Thorough, systematic examination of a large number of sections noting both presence and absence of structures and features is essential, particulary if one is reporting absence of anomalies.

- It is important that a variety of stains are used to visualize various abnormalities. On renal tissue we have a routine panel of stains including:
  1. hematoxylin and eosin (H&E)
  2. masson trichrome (stains collagen ie fibrosis/scarring)
  3. Jones silver stain (highlights the basement membranes)
  4. PAS (stains mesangial matrix as well as basement membranes)

  Were all of these stains used?
- Electron microscopy (EM) should have been performed to identify early ultastructural changes or damage to either the podocyte foot processes or basement membranes. EM is also a valuable tool to identify electron dense deposits and their specific location within the glomeruli.
- Were other organs such as the liver, lungs, pancreas etc. examined? If one organ is being re-examined, presumably in light of more modern methodologies, then this re-examination should be comprehensive and should be extended to all organs. Nephropathy may not be an isolated finding.

What is the PMRA thinking? Data evaluation reports were not available

It is hard to discern the exact reasoning behind elements of the PMRA's decision, and acceptance of the industry re-examination and retraction of previously clear statements regarding toxic effects at lower doses is particularly puzzling. The PMRA interpretation of the industry-supplied reports is contained in Data Evaluation Reports (DERs) or equivalent documents, but these were not available. The DERs provide the link between the confidential test data and the published evaluation reports and decisions. The DERs may have also explained, for instance, why the same sets of studies could support two different NOAELs, and how the PMRA came to the conclusion that no LOAELs were utilized when it appears that some might have been used.

Making the DERs or equivalent available was a key recommendation of the Reading Room Pilot Project, but this has not yet been acted upon. The DERs and equivalents should be publicly available.

Modern Scientific Standards for Review and Synthesis of Scientific and Medical Data

An essential tool for review and synthesis of scientific and medical data is a searchable references database with separate fields for information such as author, title, journal information, year and type of publication, keywords and abstract / synopsis. The reference should be linked to the full text document, and the full text document must also be searchable. If this was in place there would be no difficulty generating an annotated bibliography that may even preclude the need for a visit to the Reading Room. I have done this with the references for this submission, for the convenience of the reader and as an illustration of what is possible. Such a database greatly facilitates compilation of information and review of a particular topic. For example, complete and up to date evidence regarding exposure assessment, biological effects (e.g. endocrine effects), or disease (e.g. cancer) linked to a pesticide should be at the PMRA assessors' fingertips. An expert in information science (librarian) could routinely search publicly accessible scientific literature and update these pesticide databases. It should not be up to the public to point out relevant peer-reviewed articles, as is presently apparently the case.

Pesticide assessment could be facilitated by extracting key data into electronically accessible forms, which would allow comparison and pooling of endpoints from a variety of studies. An example of such a system is Trialstat, an Ottawa-based online system for systematic review of medical literature, that allows many researchers around the globe to work together. This is particularly of interest for older pesticides such as 2,4-D, given the vast amount of data in the publicly available literature.

"The dose makes the poison" is out of date

The 16th century writings of Paracelsus have been shortened to this statement, which I heard numerous times during my visits to the PMRA and have heard many times from pesticide proponents at public meetings. There are numerous ways in which this summation is now known to be misleading and false.

Non-monotonic dose-responses

In toxicological risk assessment the dose-response curve is assumed to be monotonic. Studies for pesticide assessment are carried out with relatively high doses of chemicals compared to what would be expected under conditions of use. However, it is now increasingly recognized that low dose effects may be different, even opposite, and at least as serious as high dose effects (some peer-reviewed studies are summarized at: http://www.environmentalhealthnews.org/sciencebackground/2007/2007-0415nmdrc.html).

Since pesticide assessment includes extrapolating a NOAEL to an acceptable human dose, manufacturers would only wish to explore animal doses that are high enough such that the use would be allowed after application of extrapolation factors. Discovery of low dose effects would mean that the pesticide could not be registered according to the current paradigm. Nevertheless, current science indicates that biologically active substances must be tested over many orders of magnitude of doses to adequately assess the entire spectrum of their effects. It is possible that a NOAEL would be measured at the nadir of a U-shaped response curve, while environmental exposures would trigger significant adverse effects. Indeed, although the data is of very poor quality, it appeared in examination of confidential test data that some effects might be more pronounced at lower doses than at higher ones. To examine this thoroughly would require considerably more time and access (e.g. to data synthesis programs and formats) than was available to me in the Reading Room. These observations were uniformly dismissed as evidence of scatter in the data rather than trends.

Adequacy of measurement endpoints

At present, registrants are obliged to provide the PMRA with in vitro genotoxicity studies. It is not considered to be genotoxic, but the genotoxicity of 2,4-D was demonstrated in vivo and in vitro, in male applicators applying only 2,4-D, and their lymphocytes.[5] Importantly, pesticide exposure may also lead to epigenetic changes. These epigenetic changes determine which DNA is going to be expressed (and how), and it is now understood that at least some forms of epigenetic variation are heritable, producing multi-generational effects.[6,7] This is not assessed, but is one reason to extend the multi-generational study.

Neurological outcomes are also difficult to assess and are sometimes not required to be fully explored,[8] although implications are serious for our society.[9] Developmental neurotoxicity was examined almost 20 years ago in crude experiments. "Confirmatory" data is being requested although it is not clear what will transpire if this data is not forthcoming. The protocol is under development presently and summary recommendations for this study are presented above. I bring to the attention of the PMRA a recent study in rats showing that exposure to as little as 10 mg/kg bw/day 2,4-D during the first post partum days produced changes in maternal behavior, serum prolactin and monoamine levels in the AcN of treated dams.[2] This is in contrast to the 12.5 and 25 mg/kg bw/day short term NOAELs incorporated in the 2,4-D re-assessment. This new information of a lower LOAEL should necessitate recalculations.

Cumulative assessments – pesticides in combination or sequence

The PCPA now requires that cumulative assessments be conducted for pesticides with a common mechanism of toxicity, and this is a work in progress for cholinesterase-inhibiting insecticides. The

groundbreaking work of York scientists further highlights the complexity of this topic. They recently demonstrated that exposure to two pesticides, had different effects on mortality of Gammarus pulex (small shrimp) depending upon the order of exposure.[10] Despite their close chemical resemblance to one another, and the fact that some have common breakdown products, the phenoxy herbicides did not undergo a cumulative assessment.

Cumulative toxicities may contribute to inconsistencies between toxicological and epidemiological studies. Although not specifically required under the PCPA, given that information from the PMRA feeds into the Drinking Water considerations, an argument could be made that the PMRA is indeed required to assess cumulatively effects of pesticides with related effects (complementary, additive, antagonistic or synergistic). Pesticides that have effects on the same type of cell or organ should be assessed in a cumulative manner.

Phenoxy herbicides were not assessed in a cumulative manner, in spite of the fact that they belong to a common class and are applied as mixtures for a synergistic killing effect. This is not because the chemicals do not have common, complementary or synergistic mechanism of toxicities, but because the mechanism(s) of toxicity are not well understood. However, materials that are chemically similar and have common mechanisms of toxicity in the target organisms may indeed share other toxic effects in non-target organisms. *The risks of pesticides of a common class should be considered to be cumulative, as should risks of pesticides affecting a common organ or biological system.*

Estimation of exposure is a major source of uncertainty in epidemiological studies, and contributes to the PMRA opinion that epidemiological studies are not reliable enough to determine whether or not present practices are protective of human health. Significantly however, estimation of human exposure is also key information that contributes to toxicological assessment. The chief improvement in "certainty" between toxicological and epidemiological studies arises from the use of homogeneous populations of animals in controlled environments as opposed to humans of mixed ages, genetics, environments, co-exposures, co-morbidities, etc. This is mentioned in support of my strong recommendation that epidemiological studies be used in pesticide assessment, or as they become available in subsequent adaptive management of hypotheses.

Animals in cages are not exposed to sporadic contaminants (e.g. 2,7-DCDD in 2,4-D), or breakdown products (e.g. dichlorophenol), so toxicology does not assess these important components of the total toxicity and outcomes of pesticide use. Only other research such as epidemiological and environmental evidence will assess these aspects. This research should be supported and carried out.

Inadequacy of extrapolation factors

Some say that using multiplicative extrapolation factors is a conservative, protective approach, but this view is not consistent with the fact that detoxification abilities of humans may vary more than 10-fold for many reasons, including genetic variability and enzyme levels, with observable consequences as a result of pesticide exposure.[11-20] Elucidation of the genomes of rats and mice led to cautions that they may be of limited utility in toxicological assessments because of enzymes for detoxification that do not exist in people. It is possible that the simplistic, historical approach is overlooking many substantial considerations that actually render it overly optimistic about pesticide safety and underestimate risk. The Swedish Chemicals Inspectorate suggests that factors greater than 10-fold may be appropriate for certain cases, and discusses expansion of intraspecies extrapolation factors into several factors.[21]

Types of toxicity

Some chemicals are toxic in ways that lead to immediate effects. For example, an acid burns the skin. Other chemicals that affect hormones or the immune system for instance may have delayed

effects that are not seen for years, or may even affect the next generation. Pesticides are also a very common initiating factor for people experiencing environmental sensitivities, and the numbers of people reporting reactions to domestic herbicide sprayings to groups such as the Allergy and Environmental Health Associations demonstrates that there is a reasonable certainty of harm, not "no harm," for some people. Establishing cause/effect relationships in the literature for delayed effects may be difficult. The two important points in this regard are that insignificant results are not proof that no effect exists, and consistency of effect, even if rather low-level, will under-estimate any effect and should be taken very seriously.

Epidemiology and Other Evidence

In any scientific endeavour, there is an allure of precision that can only be found under tightly controlled experimental conditions. Thus, toxicological investigations in genetically homogeneous animals exposed to a single chemical at a time are seen to trump observational human studies with their confounding factors and potential for biases and errors. On the other hand, medical professions dealing daily with these complex phenomena appreciate that real life has to pre-empt laboratory certitude. Somehow, this reality has to start impinging upon toxic chemical assessment.

Limitations in toxicological experimentation are extensively discussed elsewhere in this submission. It is instructive to examine recent epidemiological evidence.

Non-Hodgkin lymphoma (nHL)

A study previously pointed out to the PMRA is the 2006 report by Chiu et al. on non-Hodgkin lymphoma (nHL) with t(14;18)(q32;q21) genetic translocation (Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma).[3] This was discussed in the RVD2008-11, with the statement that this study demonstrated that people using 2,4-D were less likely to develop the subtype of nHL. This is a misleading and incorrect interpretation of Table 4, from that report, which is reproduced in part here.

Reproduced in part from Chiu *et al* (2006)[3]
Chui *et al.'s* Table 4. The association of non-Hodgkin lymphoma (NHL) among farmers associated with chemical classes of herbicides according to t(14;18) status

Herbicide use	Control subjects	t(14;18)-positive NHL vs control subjects		t(14;18)-negative NHL vs control subjects		*P* for difference†
		Case subjects	OR (95% CI)*	Case subjects	OR (95% CI)*	
Never used pesticides	229	5	1.0 (referent)	23	1.0 (referent)	NA
Phenoxyacetic acids No‡	31	4	5.0 (1.2-20.8)	4	1.5 (0.5-4.8)	0.2
Phenoxyacetic acids Yes	180	14	2.9 (1.0-8.4)	14	0.8 (0.4-1.6)	0.04
data regarding other classes of herbicides is also reported						

NA indicates not applicable.
*Odds ratios (ORs) and confidence intervals (CIs), adjusted for age (4 levels), sex, type of respondent (direct or proxy interview), and family history of cancer (yes or no).
†*P* for difference was computed from polytomous logistic regression by testing the difference of regression coefficients of the usage of pesticides corresponding to
t(14;18)-positive versus control subjects and t(14;18)-negative versus control subjects.
‡Did not use the specific chemical class of herbicides but may still have used insecticides or other chemical classes of herbicides.

In this study, the incidence of the subset of nHL with the t(14;18) translocation was increased with exposure to various types of herbicides, and reached statistical significance for the group that used phenoxy herbicides (2,4-D is in most phenoxy herbicide mixtures commonly used). The group the PMRA was referring to was the very small group that used other pesticides but not 2,4-D. This group is too small to be informative, and the exposures were diverse, so the authors did not present a direct comparison. The 95% confidence interval for the small group using other pesticides is very large and overlaps that for the group using phenoxy herbicides, so despite the claim by the PMRA, there is no statistically significant difference between the groups. The valid comparison presented in this work is between the group that used phenoxy herbicides and the group that used no pesticides whatsoever. Furthermore, elsewhere in the paper a dose-response for herbicide use is reported, that further strengthens this finding (60% of herbicide applicators used phenoxy herbicides).

In the past few years there have been several reports of cancers and other health problems linked to 2,4-D. This is not a comprehensive or systematic review, but is a few references found by searching PubMed (a free online medical search service), provided to illustrate deficiencies in the PMRA literature assimilation and consideration, and to provide a few examples to illustrate ample reason for increased prudence in the use of this chemical. First author searches were used on the reference lists provided by the PMRA.

"Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies" by Miligi et al was not in the PMRA reference list.[22] They report that in total, 1925 cases and 1232 controls were interviewed in the nine agricultural areas. Increased risk was observed for some specific classes of pesticides. Furthermore, a nonstatistically significant increased risk of NHL was observed for subjects who were exposed to phenoxy herbicides not using protective equipment and a significantly increased risk for exposure to 2,4-dichlorophenoxy acetic acid (2,4-D).

"Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988-2001" by Mills et al is not on a PMRA references list.[23] However, it was discussed and dismissed as inconclusive by the PMRA. Not only does it show a statistically significant increased risk, the authors report a dose-dependent increase, which is an indicator of much stronger evidence. The authors report that employment in farms using 2,4-D was associated with an increased risk of NHL.

"Agricultural exposures and gastric cancer risk in Hispanic farm workers in California" by Mills et al is not on the PMRA references list.[24] The authors report that working in areas with high use of the phenoxyacetic acid herbicide 2,4-D was associated with gastric cancer (OR=1.85; 95% CI=1.05-3.25) … These findings may have larger public health implications especially in those areas of the country where these pesticides are heavily used and where they may be found in the ambient atmosphere.

The author SH Swan is not on the PMRA references list. In "Semen quality in relation to biomarkers of pesticide exposure"[25] they report that the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and metolachlor were also associated with poor semen quality in some analyses…

McDuffie et al. wrote a series of reports on nHL and pesticides, not all of which are referenced by the PMRA.[26-29] They report on exposure, confounding, and on effects of individual chemicals. "Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CI, 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81) … We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors."

A recent paper, "Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies" was not considered by the PMRA.[30] The authors report that after

stratification by chemical class, consistent increases in the risk of leukaemia were found in all groups but statistical significance was found only for phenoxy herbicides unlikely to have been contaminated with dioxins and furans. This last finding appears equivocal in view of the existing literature. The separate analysis conducted on leukaemias from the myeloid lineage showed the highest relative risk (6.99; 95% CI 1.96-24.90).

"Atopy, exposure to pesticides and risk of non-Hodgkin lymphoma" by Vadkic *et al*. [31] is not in a PMRA reference list. It is important because it deals with previously postulated confounding factors. "The OR for NHL with substantial pesticide exposure and any history of asthma was 3.07 (95% CI 0.55-17.10) and with substantial pesticide exposure and no asthma history it was 4.23 (95% CI 1.76-10.16). The p-value for interaction was 0.29. A similar pattern of risk was observed for each of the pesticide subtypes; for asthma at various times of life; for hay fever, eczema, food allergy and any atopy, in men only and for follicular lymphomas only. Although this study had limited power, the findings do not suggest modification of the association between pesticide exposure and NHL risk by asthma or atopic disease more generally."

"Metabolic gene variants and risk of non-Hodgkin's lymphoma" is not on the PMRA bibliographic list, although a 2003 paper by De Roos et al. was referenced[32,33] This work adds to literature regarding health outcomes and toxin metabolism, including a Canadian study of alleles in children with leukemia. The lead author is a member of the Pest Management Advisory Committee, but "Risk of childhood leukemia associated with exposure to pesticides and with gene polymorphisms" was also not referenced by the PMRA.[20]

Childhood Cancer

In the PACR2005-01, the Scientific Advisory Committee recommended that childhood cancer related to exposure to 2,4-D merited more study. There has been no evidence of this work by the PMRA. However, in 2007 Dr. Infante-Rivard *et al.* published "Pesticides and childhood cancer: an update of Zahm and Ward's 1998 review," stating "one can confidently state that there is at least some association between pesticide exposure and childhood cancer.[34] However, an unambiguous mechanistic cause-and-effect relationship between pesticide exposure and childhood cancer was not demonstrated in these studies, and modifying factors such as genetic predisposition, rarely considered in the reviewed studies, likely play an important role. While the time window of exposure may be a crucial determinant for biological effects associated with pesticide exposure on children, studies have not contributed definitive information on the most vulnerable period. Accurate exposure assessment remains a challenge; future epidemiological studies need to assess gene-environment interactions and use improved exposure measures, including separate parental interviews, specific pesticide exposure questions, and semiquantitative exposure measures that can be used to confirm information obtained through questionnaires." In this situation, it is the role of the PMRA to minimize children's exposures to the pesticides examined in such studies, and 2,4-D is very commonly used.

Non-Hodgkin lymphoma

Dr. Richard van der Jagt is one of Canada's leading clinicians and researchers in lymphoma and leukemia. He is convinced that 2,4-D is linked to the rapidly increasing numbers of patients he sees in his clinics. He writes, "I am a full time hematologist, a physician caring for people with cancers such as leukemia and lymphoma, at the Ottawa Hospital. Since I started practicing 18 years ago, the incidence of lymphoma has dramatically increased. I am also the Chair and founder of the Canadian Leukemia Studies Group, a collaboration of over 30 academic medical centers researching these cancers.

"Non-Hodgkin lymphoma is commonly linked to pesticides, particularly to herbicides such as 2,4-D. According to the National Cancer Institute's website, which describes and tracks cancer across Canada:

- There has been a total of 1700 deaths in Canada due this year due to NHL;
- In both males and females, incidence rates increased approximately 50% between 1978 and the late 1990s.

"I have experienced this increased incidence in my own practice as we are now over-run with lymphoma patients. Many of them have the non-Hodgkin lymphoma most closely linked with pesticide exposure, as reported in top quality, high impact and peer reviewed journals, that set a high bar before accepting papers for publication." Dr. van der Jagt pointed to some of the articles cited above.

Numerous authors report increased incidence of cancers with use of herbicides on lawns, as reviewed by Bassil et al.[35] 2,4-D is the most common herbicide used on lawns. Recently, breast cancer, the most common cancer in women, was linked to herbicides used on lawns.[36] Non-cancer endpoints are also linked strongly and repeatedly to phenoxy herbicides and 2,4-D in particular.[37] I will not exhaustively review this issue again,[1] but at the present, the value and importance of these reports to pesticide regulation appears to be zero. Somehow, this information has to be taken into account.

The above is not exhaustive, but is intended to indicate serious weaknesses in the PMRA selection and discussion of scientific literature. It also appears that study citation was driven by outside supplies of information; not by independent searching for information. Studies cited in the PACR2005-01 were almost uniformly in favour of 2,4-D, while less favourable studies were cited in subsequent publications as they were pointed out by the public. However, the lack of comprehensive study citation in later reports makes this is difficult to establish unequivocally.

Within studies of phenoxy herbicides and 2,4-D, there is remarkable consistency of effects, and in some cases even a measured dose-dependent response. This information has to contribute to the assessment.

Other forms of evidence, such as hormonal activity that would affect development, including for example estrogenic activity that would promote breast cancer,[38] or androgenic activity that would promote prostate cancer,[39] were also neither referenced nor considered by the PMRA.

Dioxins

There is no dispute that dioxins contaminate 2,4-D. Indeed, according to Environment Canada, phenoxy herbicides are the largest source of lower chlorinated dioxins in the Canadian environment.[40]

There is an economic incentive to produce herbicides with higher levels of contamination because dioxins are produced in greater quantity at higher reactor temperatures; the same conditions under which production is more rapid and conversion from feed stocks more complete. However, measures proposed by the PMRA to obtain contaminant information from the applicants will not be reliably informative because manufacturers monitor reactor temperature and dioxin contamination, so samples and data are readily selected by the applicants and may not be representative of products in use in Canada. The PMRA should review all production contaminant data from companies over the past perhaps 5 years, and also conduct its own tests of off-the-shelf phenoxy herbicides. Research is urgently needed on the health impacts of lower chlorinated dioxins, as well as their prevalence in the Canadian population and environment, as well as foods.

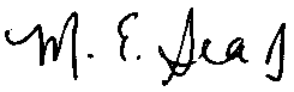
Dioxins with 4 or more chlorine atoms are well known to be toxic, and are highly regulated and monitored. The health impacts of lower chlorinated dioxins, in particular 2,7-dichlorodiphenyl-p-dioxin (2,7-DCDD) have only been reported in a couple of studies[41,42] (in contrast to thousands of studies of the dioxin considered to be most toxic, 2,3,7,8-TCDD). Contrary to claims made in the RVD2008-11by the PMRA that authorities in other jurisdictions do not consider 2,7-DCDD to be toxic, the Agency for Toxic Substances and Disease Registry in the United States considers it to be "equipotent" to the most toxic dioxin, 2,3,7,8-TCDD, in these tests of immune suppression.[43] Immune suppression is important in development of any cancer, and in particular haematological malignancies such as nHL.

Acknowledgements and Conflicts of Interest

I have received no financial support for visiting the Reading Room or preparation of this document. I paid my own parking, and I was treated to one cup of coffee. I have no ongoing financial support for my work, so lost money in this endeavour. I have no reason to believe that a change in the 2,4-D registration will bring me any financial gain, but I do hope for healthier children in a better environment.

I thank numerous colleagues with whom I have corresponded over the years. I did not solicit permission to name them all, but they know who they are and will recognise where I have shamelessly borrowed and expanded upon their intelligent thoughts.

Respectfully submitted,



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August 15, 2008

Appendix A

The following paper is provided as a pdf, along with the electronic submission.

Sears M, Walker CR, van der Jagt RHC, Claman P. Pesticide Assessment: Protecting Public Health on the Home Turf. *Paediatr Child Health.* 2006;11:229-234

Appendix B

June 18th, 2008 email from the PMRA describing reports for various NOAELs.

Q1. Which reports were relied upon to identify the various NOAELs

The studies/NOAELs used in the risk assessment are identified in the PACR 2007-06, Table 1 Toxicology Endpoints Used in the 2,4-D Agriculture Risk Assessment (page 68). To help you find the studies in the reading room, we have listed the PMRA document # associated with each study below. When you come in on Friday to inspect the data, I will provide you with a marked-up reference list to identify which studies produced NOAELs. Not all NOAELs are used for endpoint selection; we default to the lowest NOAEL for a particular endpoint of concern and relevant exposure period.

There were many forms of 2,4-D (acid, DMA, EHE, BEE, IPA, TIPA etc), therefore there are several studies to support bridging the data to the acid database. The PMRA looked at the studies as a whole to ensure that there was qualitative/quantitative toxicological equivalence. In other words, we compared the studies to see if they were alike, with the same effects happening at certain dose levels. This is how we determined that DEA was not toxicologically equivalent to the acid.

Acute Dietary for females 13-50: NOAEL of 25 mg/kg bw/d from a rat developmental study (PMRA# 1291538 (BEE); #1292201(IPA); #1311889(DEA); #1370428 (acid); #1373794 (EHE); #1379309 (DMA); #1417864 (TIPA)).

Acute Dietary for general population: NOAEL of 75 mg/kg bw/d from an acute rat neurotoxicity study (PMRA# 1370424).

Chronic Dietary: NOAEL of 5 mg/kg bw/d from the chronic rat study (PMRA# 1370407).

Short-term exposure (>1 day - 6 months) females 13-50: NOAEL of 30 mg/kg bw/d (PMRA# 1370430 (acid); 1373796 (EHE); 1379311(DMA)) or 10 mg/kg bw/d (PMRA# 1291540 (BEE); 1292203 (IPA) and 1417865 (TIPA)) from several rabbit developmental studies. Reason for the two assessments: when comparing the studies as a whole, there was qualitative tox equivalence, but the effects were happening at lower doses with the BEE, IPA and TIPA forms. Thus, the risk assessment was split. This is explained in both the lawn and turf and the agricultural assessments.

Short-term exposure (>1 day - 6 months) general population): 12.5 mg/kg bw/d in a rat developmental study (PMRA# 1291538 (BEE); 1292201(IPA); 1311889(DEA); 1370428 (acid); 1373794 (EHE); 1379309 (DMA); 1417864 (TIPA)).

Aggregate (1-7 day) Females 13-50, NOAEL of 30 mg/kg bw/d (PMRA# 1370430 (acid); 1373796 (EHE); 1379311(DMA)) or 10 mg/kg bw/d (PMRA# 1291540 (BEE); 1292203 (IPA) and 1417865 (TIPA)) from several rabbit developmental studies.

Aggregate (1-7 day) general population: 12.5 mg/kg bw/d in a rat developmental study (PMRA# 1291538 (BEE); 1292201(IPA); 1311889(DEA); 1370428 (acid); 1373794 (EHE); 1379309 (DMA); 1417864 (TIPA)).

Q2. Confirmatory data

Please see the REV2006-11, page 20-22, comments 7.1 and 7.3

The PMRA used both published and unpublished studies to support their request for confirmatory data. Currently the 2,4-D database has a 2-generation reproduction study that showed no effects at dose levels up to 20 mg/kg bw/d (PMRA # 1370418). However, because of a dosing error, the high dose of 80 mg/kg bw/d was actually 115 mg/kg bw/d and this dose level caused toxicity. With the current study on file, we were able to set an offspring NOAEL at 20 mg/kg bw/d. The current ADI is 1176x lower than this NOAEL.The PMRA requested an updated study, that is without a dosing error.

The triggers for the developmental neurotoxicity study were retinal damage and effects on forelimb grip strength in adult rats (PMRA #1370426, chronic neurotoxicity study) at high dose levels. These effects had a NOAEL of 75 mg/kg bw and thus, the ADI is 4411x lower than this NOAEL.

Published studies involving intraperitoneal and subcutaneous administration of 2,4-D acid to pregnant rats, as well as oral exposure to pups through mother's milk during postnatal days 15-25, resulted in myelin deficiency in the central nervous system of pups. In addition, a combination of pre and post-natal exposure induced a delay in the development of surface righting reflex, geotaxic response and hindlimb support in rat pups, which correlated with alterations in the development of the monoamine systems in the brain of these rats as adults (Bortolozzi et al., 1999, 2003; Duffard et al.,, 1995, 1996; Rosso et al.,, 1997, 2000; Sturtz et al.,, 2000). Although these effects were observed at much higher dose levels relative to the doses producing effects in short and long-term studies, these findings may be an indication of offspring sensitivity after exposure to 2,4-D during pre- and post-natal development. Although we couldn't set a NOAEL (study not conducted in a way to do so), a dose of 70 mg/kg bw/d was showing effects. The ADI is 4117x lower than this effect.

As explained in the response to comments (REV2006-11), additional safety factors were added to the assessment to ensure that an adequate margin of safety was attained to these NOAELs and potential effects. Even with the PMRA taking the most conservative assumptions, the risks were found to be acceptable. Therefore, the additional confirmatory studies will be used to further refine the assessment.

References, annotated with abstracts

(1) Sears M, Walker CR, van der Jagt RHC, Claman P. Pesticide Assessment: Protecting Public Health on the Home Turf. *Paediatr Child Health.* 2006;11:229-234.
Abstract: Pesticide regulation is examined in the context of Health Canada's Pest Management Regulatory Agency's assessment of the chlorophenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) for turf. 2,4-D is the most common herbicide used to kill weeds in grass. The medical literature does not uniformly indicate harms from herbicides. However, the balance of epidemiological research suggests that 2,4-D can be persuasively linked to cancers, neurological impairment and reproductive problems. These may arise from 2,4-D itself, from breakdown products or dioxin contamination, or from a combination of chemicals. Regulators rely largely on toxicology, but experiments may not replicate exposures from 2,4 D application to lawns because environmental breakdown products (eg, 2,4-dichlorophenol) may not accumulate and selected herbicides are possibly less contaminated. Dioxins are bioaccumulative chemicals that may cause cancer, harm neurological development, impair reproduction, disrupt the endocrine system and alter immune function. No dioxin analyses were submitted to the Pest Management Regulatory Agency, and the principal contaminants of 2,4-D are not among the 17 congeners covered in pesticide regulation. Independent assessment of all dioxins is needed, in tissues and in the environment. The 2,4-D assessment does not approach standards for ethics, rigour or transparency in medical research. Canada needs a stronger regulator for pesticides. Potentially toxic chemicals should not be registered when more benign solutions exist, risks are not clearly quantifiable or potential risks outweigh benefits. Until landscaping pesticides are curtailed nationally, local bylaws and Quebec's Pesticide Code are prudent measures to protect public health. Physicians have a role in public education regarding pesticides.

(2) Sturtz N, Deis RP, Jahn GA, Duffard R, Evangelista de Duffard AM. Effect of 2,4-dichlorophenoxyacetic acid on rat maternal behavior. *Toxicology.* 2008;247:73-79.
Abstract: Exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) has several deleterious effects on the nervous system such as alterations in the concentrations of neurotransmitters in the brain and/or behavioral changes, myelination rate, ganglioside pattern [Bortolozzi, A., Duffard, R., Antonelli, M., Evangelista de Duffard, A.M., 2002. Increased sensitivity in dopamine D(2)-like brain receptors from 2,4-dichlorophenoxyacetic acid (2,4-D)-exposed and amphetamine-challenged rats. Ann. N.Y. Acad. Sci. 965, 314-323; Duffard, R., Garcia, G., Rosso, S., Bortolozzi, A., Madariaga, M., DiPaolo, O., Evangelista de Duffard, A.M., 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol. Teratol. 18, 691-696; Evangelista de Duffard, A.M., Orta, C., Duffard, R., 1990. Behavioral changes in rats fed a diet containing 2,4-dichlorophenoxyacetic butyl ester. Neurotoxicology 11, 563-572; Evangelista de Duffard, A.M., Bortolozzi, A., Duffard, R.O., 1995. Altered behavioral responses in 2,4-dichlorophenoxyacetic acid treated and amphetamine challenged rats. Neurotoxicology 16, 479-488; Munro, I.C., Carlo, G.L., Orr, J.C., Sund, K., Wilson, R.M. Kennepohl, E. Lynch, B., Jablinske, M., Lee, N., 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. J. Am. Coll. Toxicol. 11, 559-664; Rosso et al., 2000], and its administration to pregnant and lactating rats adversely affects litter growth and milk quality. Since normal growth of the offspring depends on adequate maternal nursing and care, we evaluated the effect of 2,4-D on rat maternal behavior as well as the dam's monoamine levels in arcuate nucleus (AcN) and serum prolactin (PRL) levels. Wistar dams were exposed to the herbicide through the food from post partum day (PPD) 1 to PPD 7. Dams were fed either with a 2,4-D treated diet (15, 25 or 50mg 2,4-D/kg/daybw) or with a control diet. We observed that maternal nesting behavior was not modified by 2,4-D treatment. However, mother-pup interactions, specially the nursing behavior, were altered. Retrieval, crouching and licking of pups were reduced or suspended after 2,4-D treatment. We also observed an increase in the latency of retrieval and crouching in the dams treated with the herbicide. Dams showed movement along cage peripheries, food consumption during the light phase and high self-grooming. In addition of the deficits observed in maternal behavior parameters, increased catecholamine levels and a drastic decrease in indolamine levels in the AcN of treated dams were determined. Serum PRL levels were also diminished by 62%,

68% and 70% with respect to control dams in the 15, 25 and 50mg 2,4-D/kgbw treated dams, respectively. In conclusion, exposure to 2,4-D during the first post partum days produced changes in maternal behavior, serum prolactin and monoamine levels in the AcN of treated dams

(3) Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma. *Blood.* 2006;108:1363-1369.
Abstract: Pesticides have been specifically associated with the t(14;18)(q32;q21) chromosomal translocation. To investigate whether the association between pesticides and risk of non-Hodgkin lymphoma (NHL) differs for molecular subtypes of NHL defined by t(14; 18) status, we obtained 175 tumor blocks from case subjects in a population-based case-control study conducted in Nebraska between 1983 and 1986. The t(14;18) was determined by interphase fluorescence in situ hybridization in 172 of 175 tumor blocks. We compared exposures to insecticides, herbicides, fungicides, and fumigants in 65 t(14;18)-positive and 107 t(14;18)-negative case subjects with those among 1432 control subjects. Multivariate polytomous logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Compared with farmers who never used pesticides, the risk of t(14;18)-positive NHL was significantly elevated among farmers who used animal insecticides (OR = 2.6; 95% CI, 1.0-6.9), crop insecticides (OR = 3.0; 95% CI, 1.1-8.2), herbicides (OR = 2.9; 95% CI, 1.1-7.9), and fumigants (OR = 5.0; 95% CI, 1.7-14.5). None of these pesticides were associated with t(14;18)-negative NHL. The risk of t(14;18)-positive NHL associated with insecticides and herbicides increased with longer duration of use. We conclude that insecticides, herbicides, and fumigants were associated with risk of t(14;18)-positive NHL but not t(14;18)-negative NHL. These results suggest that defining subsets of NHL according to t(14;18) status is a useful approach for etiologic research

(4) Ohshika H, Endo J, Takemura H, Tanaka M. A possible role of alpha adrenergic receptors in salivation induced by isoproterenol in mice. *Jpn J Pharmacol.* 1978;28:650-652.

(5) Holland NT, Duramad P, Rothman N et al. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. *Mutat Res.* 2002;521:165-178.
Abstract: Widespread use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments (P=0.052). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group ($P<0.01$), in accordance with the in vitro finding of increased RI at low doses

(6) Anway MD, Skinner MK. Epigenetic Transgenerational Actions of Endocrine Disruptors. *Endocrinology.* 2006;147:s43-s49.
Abstract: Endocrine disruptors have recently been shown to promote an epigenetic transgenerational phenotype involving a number of disease states (e.g. male infertility). The anti-androgenic fungicide vinclozolin was found to act transiently at the time of embryonic sex determination to promote in the F1 generation a spermatogenic cell defect and subfertility in the male. When the animals were allowed to age up to 1 yr, a number of other disease states developed. This phenotype was transferred through the

male germ line to all subsequent generations analyzed (F1-F4). The ability of an environmental factor (i.e. endocrine disruptor) to promote an epigenetic transgenerational phenotype impacts the potential hazards of environmental toxins, mechanisms of disease etiology, and evolutionary biology. The biological importance of the epigenetic actions of environmental agents is reviewed in the context of the primordial germ cell and development of epigenetic transgenerational phenotypes

(7) Crews D, McLachlan JA. Epigenetics, Evolution, Endocrine Disruption, Health, and Disease. *Endocrinology.* 2006;147:s4-10.
Abstract: Endocrine-disrupting chemicals (EDCs) in the environment have been linked to human health and disease. This is particularly evident in compounds that mimic the effects of estrogens. Exposure to EDCs early in life can increase risk levels of compromised physical and mental health. Epigenetic mechanisms have been implicated in this process. Transgenerational consequences of EDC exposure is also discussed in both a proximate (mechanism) and ultimate (evolution) context as well as recent work suggesting how such transmission might become incorporated into the genome and subject to selection. We suggest a perspective for exploring and ultimately coming to understand diseases that may have environmental or endocrine origins

(8) Welch DA, Christenson D, Penley L, Chan P, Scoggins P, Shapiro S, Coryell M, Smith W, and O'Grady J. Letter from the National Treasury employees Union to Stephen L. Johnson, Administrator, U.S. Environmental Protection Agency. Public Employees for Environmental Responsibility . 24-5-2006. http://www.peer.org/docs/epa/06_25_5_union_ltr.pdf
Abstract: With regard to lack of neurotoxicity testing of 20 neurotoxic pesticides, which is not being carried out, "We Local Presidents of EPA Unions representing scientists, risk managers, and related staff, are writing to express our concern that EPA could betray the public trust by violating the intention of the Food Quality Protection Act (FQPA) to protect the Nation's infants, children, and susceptible subpopulations, unless the Agency adheres to principles of scientific integrity and sound science in the pesticide tolerance reassessments it is undertaking."

(9) Colborn T. A case for revisiting the safety of pesticides: a closer look at neurodevelopment. *Environ Health Perspect.* 2006;114:10-17.
Abstract: The quality and quantity of the data about the risk posed to humans by individual pesticides vary considerably. Unlike obvious birth defects, most developmental effects cannot be seen at birth or even later in life. Instead, brain and nervous system disturbances are expressed in terms of how an individual behaves and functions, which can vary considerably from birth through adulthood. In this article I challenge the protective value of current pesticide risk assessment strategies in light of the vast numbers of pesticides on the market and the vast number of possible target tissues and end points that often differ depending upon timing of exposure. Using the insecticide chlorpyrifos as a model, I reinforce the need for a new approach to determine the safety of all pesticide classes. Because of the uncertainty that will continue to exist about the safety of pesticides, it is apparent that a new regulatory approach to protect human health is needed

(10) Ashauer R, Boxall AB, Brown CD. New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environ Sci Technol.* 2007;41:1480-1486.
Abstract: Aquatic nontarget organisms are exposed to fluctuating concentrations or sequential pulses of contaminants, so we need to predict effects resulting from such patterns of exposure. We present a process-based model, the Threshold Damage Model (TDM), that links exposure with effects and demonstrate how to simulate the survival of the aquatic invertebrate Gammarus pulex. Based on survival experiments of up to 28 days duration with three patterns of repeated exposure pulses and fluctuating concentrations of two pesticides with contrasting modes of action (pentachlorophenol and chlorpyrifos) we evaluate the new model and compare it to two approaches based on time-weighted averages. Two models, the Threshold Damage Model and the time-weighted averages fitted to pulses, are able to simulate the observed survival (mean errors 15% or less, r2 between 0.77 and 0.96). The models are

discussed with respect to their theoretical base, data needs, and potential for extrapolation to different scenarios. The Threshold Damage Model is particularly useful because its parameters can be used to calculate recovery times, toxicokinetics are separated from toxicodynamics, and parameter values reflect the mode of action

(11) McKeown-Eyssen G, Baines C, Cole DE et al. Case-control study of genotypes in multiple chemical sensitivity: CYP2D6, NAT1, NAT2, PON1, PON2 and MTHFR. *Int J Epidemiol.* 2004;33:971-978.
Abstract: BACKGROUND: Impaired metabolism of toxic chemicals is a postulated mechanism underlying multiple chemical sensitivity (MCS). Because genetic variation alters the rate of chemical metabolism, this study was designed to determine if MCS cases differed from controls for genetic polymorphisms in drug-metabolizing enzymes. METHODS: Female Caucasian participants (203 cases and 162 controls) were drawn from a larger case-control study based on a reproducible and validated case definition. Common polymorphisms for CYP2D6, NAT1, NAT2, PON1, and PON2 were genotyped. RESULTS: Comparing cases and controls, significant differences were found in genotype distributions for CYP2D6 (P = 0.02) and NAT2 (P = 0.03). Compared with the referent homozygous inactive (CYP2D6) or slow (NAT2) metabolizers, the odds for being CYP2D6 homozygous active (OR = 3.36, P = 0.01) and NAT2 rapid (OR = 4.14, P = 0.01) were significantly higher in cases than controls. The odds for being heterozygous for PON1-55 (OR = 2.05, P = 0.04) and PON1-192 (OR = 1.57, P = 0.04) were also significantly higher in cases. CONCLUSIONS: A genetic predisposition for MCS may involve altered biotransformation of environmental chemicals. The CYP2D6 enzyme activates and inactivates toxins; the NAT2 enzyme bioactivates arylamines to protein-binding metabolites. A gene-gene interaction between CYP2D6 and NAT2 suggested that rapid metabolism for both enzymes may confer substantially elevated risk (OR = 18.7, P = 0.002). Our finding parallels others' observation of a link between PON1 heterozygosity and neurological symptoms in Gulf War syndrome. This first demonstration of genetic variation in drug-metabolizing enzymes in association with MCS requires replication. However, it suggests new research directions on genetically variable toxin pathways that might be important in MCS

(12) Badawi AF, Cavalieri EL, Rogan EG. Effect of chlorinated hydrocarbons on expression of cytochrome P450 1A1, 1A2 and 1B1 and 2- and 4-hydroxylation of 17beta-estradiol in female Sprague-Dawley rats. *Carcinogenesis.* 2000;21:1593-1599.
Abstract: Chlorinated hydrocarbons (CHCs) are environmental contaminants that bioaccumulate and hence are detected in human tissues. Epidemiological evidence suggests that the increased incidence of a variety of human cancers, such as lymphoma, leukemia and liver and breast cancers, might be attributed to exposure to these agents. The ability of CHCs to disrupt estrogen homeostasis is hypothesized to be responsible for their biological effects. The present study examined the effect of CHCs on the expression of cytochrome P450 (CYP)1A1, CYP1A2 and CYP1B1 mRNAs and the consequent 2- and 4-hydroxylation of 17beta-estradiol (E(2)) in female Sprague-Dawley rats. Animals were administered a single dose of the LD(50) of 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD) (25 microg/kg), 2, 4-dichlorophenoxyacetic acid (2,4-D) (375 mg/kg) and dieldrin (DED) (38 mg/kg) by gavage. Seventy-two hours after treatment, increased expression of CYP1A1, CYP1A2 and CYP1B1 was observed in the liver, kidney and mammary tissue. Since CYP1A and CYP1B1 are the major enzymes catalyzing 2- and 4-hydroxylation of E(2), respectively, the effect of these CHCs on the metabolism of E(2) was investigated in rat tissues. Formation of 2- and 4-catechol estrogens was increased in a tissue-specific manner in response to treatment. TCDD was the most potent inducer for CYP1 enzyme mRNA and for the 2- and 4-hydroxylation of E(2). 2,4-D and DED induced similar responses, but less than that of TCDD. These results suggest that induction of CYP1 family enzymes and consequent increases in estrogen metabolism by CHCs in target tissues may be factors contributing to the biological effects associated with exposure to these agents

(13) Gonzalez FJ. The study of xenobiotic-metabolizing enzymes and their role in toxicity in vivo using targeted gene disruption. *Toxicol Lett.* 1998;102-103:161-6.:161-166.
Abstract: Most of the chemicals that cause toxicity in animals are metabolized and this metabolism can

either increase or decrease the extent of toxicity. A large number of enzymes are involved in the metabolism of xenobiotics. Cytochromes P450 are among the most important and these enzymes are primarily involved in metabolic activation through oxidative metabolism. Transferases, including the glutathione S-transferases, N-acetyltransferases, UDP-glucuronosyltransferases, microsomal and cytosolic epoxide hydrolases, and NAD(P)H quinone oxidoreductase are also significant in xenobiotic metabolism and can play a role in chemical sensitivities. Polymorphisms in P450s and transferases have been found in experimental animals and humans in which a certain segment of the population, usually greater than 1%, are lacking expression of a particular enzyme. In humans, polymorphisms have been associated with adverse drug reactions but have not been shown to cause any serious developmental or physiological defects thus suggesting that in mammals, xenobiotic-metabolizing enzymes may only be required for metabolism of foreign chemicals and have no other critical role. To determine the roles of xenobiotic-metabolizing enzymes in mammalian development and physiological homeostasis, and in sensitivities to chemical toxicity and carcinogenesis, targeted gene disruption was carried out to produce gene knockout mice. Several lines of mice were produced and characterized and these are discussed

(14) Cole TB, Jampsa RL, Walter BJ et al. Expression of human paraoxonase (PON1) during development. *Pharmacogenetics.* 2003;13:357-364.
Abstract: BACKGROUND: Paraoxonase (PON1), a HDL-associated enzyme, protects against toxicity from specific organophosphorus compounds and oxidized lipids. Common polymorphisms in the PON1 gene have been identified and characterized in the coding region, 5' regulatory region and 3' UTR. The Q192R coding region polymorphism determines substrate-dependent differences in catalytic efficiency of hydrolysis. The -108CT polymorphism in the 5' regulatory region has a significant effect on PON1 expression, with the -108C allele expressing on average twice the level of plasma PON1 as the -108T allele. In addition to the effects of regulatory and coding region polymorphisms on PON1 levels and activity, plasma PON1 levels are also developmentally regulated. Since PON1 levels are important in determining resistance to specific organophosphorus compounds, the time course of appearance of PON1 in newborns is of great interest. RESULTS: We report here that PON1 levels plateau between 6 to 15 months of age, and that variability in the age at which PON1 levels plateau is quite variable among individuals. In mice and rats, plasma PON1 activity reaches a plateau at 3 weeks of age. In mice that lack endogenous PON1, human transgenes encoding either PON1(Q192) or PON1(R192) under the control of the human PON1 regulatory sequences exhibited a similar time course of expression as that seen in wild-type mice, indicating conservation of the developmental regulatory elements between mouse and human PON1

(15) Costa LG, Cole TB, Furlong CE. Polymorphisms of paraoxonase (PON1) and their significance in clinical toxicology of organophosphates. *J Toxicol Clin Toxicol.* 2003;41:37-45.
Abstract: Paraoxonase (PON1) is an HDL-associated enzyme capable of hydrolyzing multiple substrates, including several organophosphorous insecticides and nerve agents, oxidized lipids, and a number of drugs or pro-drugs. Several polymorphisms in the paraoxonase (PON1) gene have been described, which have been shown to affect either the catalytic efficiency of hydrolysis or the expression level of PON1. This review discusses the relevance of these polymorphisms for modulating sensitivity to organophosphorous compounds. Animal studies characterizing the PON1 polymorphisms have demonstrated the relevance of PON1 in modulating OP toxicity and have indicated the importance of an individual's PON1 status (i.e., genotype and phenotype taken together) rather than genotyping alone. Nevertheless, direct confirmation in humans of the relevance of PON1 status in conferring susceptibility to OP toxicity is still elusive. Recent studies examining the involvement of PON1 status in determining OP susceptibility of Gulf War veterans, sheep dippers, and individuals poisoned with chemical warfare agents represent a step in the right direction, but more studies are needed, with better documentation of both the level of exposure and the consequences of exposure

(16) Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol.* 2005;69:541-550.
Abstract: Paraoxonase 1 (PON1) is a serum enzyme closely associated with high density lipoprotein

(HDL). PON1 hydrolyzes several organophosphorus compounds used as insecticides, as well as nerve agents; it metabolizes toxic oxidized lipids associated with both low density lipoprotein (LDL) and HDL; and it can hydrolyze a number of lactone-containing pharmaceutical compounds, inactivating some, while activating others. Serum PON1 activity in a given population can vary by 40-fold. Though most of this variation can be explained by polymorphisms in the coding region (Q192R) and the 5' regulatory region (T-108C), modulation of PON1 by a variety of other factors should be taken into account, including other polymorphisms recently discovered but not yet characterized. This paper examines the major factors (environmental chemicals, drugs, smoking, alcohol, diet, age, disease conditions) that have been shown to modulate PON1 activity in either direction. As PON1 plays a protective role in organophosphate toxicity, and, because of its antioxidant capacity, in cardiovascular disease, a better understanding of how PON1 can be modulated by environmental factors has potential toxicological and clinical consequences

(17) Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. *Chem Biol Interact.* 1999;119-120:429-38.:429-438.
Abstract: In human populations, serum paraoxonase (PON1) exhibits a substrate dependent polymorphism. The Arg192 isoform hydrolyzes paraoxon rapidly but diazoxon, soman and especially sarin slowly. On the other hand, the Gln192 isoform hydrolyzes paraoxon slowly, but diazoxon, soman and sarin more rapidly than the Arg192 isoform. Our experiments with a mouse model system have convincingly shown that PON1 plays a major role in the detoxication of organophosphate (OP) compounds processed through the P450/PON1 pathway. Recent studies have also shown that PON1 plays an important role in the metabolism of oxidized lipid compounds. Currently, there is an effort underway to identify genes and polymorphisms that play an important role in 'environmental susceptibility'. The PON1 polymorphism has been cited as a prime example of such a genetic polymorphism. The advent of the polymerase chain reaction (PCR) for DNA amplification with improvements, modifications and automation has provided a very convenient way to do individual genotyping. It is tempting to set up large scale PCR analyses of populations to determine individuals at risk for environmental exposures affected by the PON1 polymorphism. In fact, a number of such studies have already been carried out in examining the relationship of the PON1 polymorphism to vascular disease. We advocate the use of a high throughput two-dimensional enzyme assay that provides both PON1 genotype and phenotype (PON1 status). The high level of variation of gene expression within each genetic class in humans, together with our animal model studies indicate that it is very important to determine PON status as opposed to PON1 genotype alone. Experiments in rats and mice have shown that injection of PON1 purified from rabbit serum by the i.v., i.p. or i.m. route, significantly increases PON1 activities in rodents' plasma. Under these conditions, the acute toxicity (assessed by the degree of acetylcholinesterase inhibition) of paraoxon and chlorpyrifos oxon is significantly decreased, compared to control animals. Protection is maximal when PON1 is administered before the OPs, but still occurs when PON1 is utilized as a post-exposure treatment. Furthermore, protection by PON1 is also provided toward the parent compound chlorpyrifos. Pon1-knockout mice display a much greater sensitivity to chlorpyrifos oxon toxicity than wild mice. However, the acute toxicity of guthion, which is not a substrate for PON1, does not differ between knockout and wild mice. These observations underline the importance of considering both genetic variability of enzyme isoform as well as enzyme level (PON1 status) and the developmental time course of appearance of PON1 in developing risk assessment models

(18) Furlong CE, Cole TB, Walter BJ et al. Paraoxonase 1 (PON1) status and risk of insecticide exposure. *J Biochem Mol Toxicol.* 2005;19:182-183.

(19) Searles NS, Mueller BA, De Roos AJ, Viernes HM, Farin FM, Checkoway H. Risk of brain tumors in children and susceptibility to organophosphorus insecticides: the potential role of paraoxonase (PON1). *Environ Health Perspect.* 2005;113:909-913.
Abstract: Prior research suggests that childhood brain tumors (CBTs) may be associated with exposure to pesticides. Organophosphorus insecticides (OPs) target the developing nervous system, and until

recently, the most common residential insecticides were chlorpyrifos and diazinon, two OPs metabolized in the body through the cytochrome P450/paraoxonase 1 (PON1) pathway. To investigate whether two common PON1 polymorphisms, C-108T and Q192R, are associated with CBT occurrence, we conducted a population-based study of 66 cases and 236 controls using DNA from neonatal screening archive specimens in Washington State, linked to interview data. The risk of CBT was nonsignificantly increased in relation to the inefficient PON1 promoter allele [per PON1(-108T) allele, relative to PON1(-108CC): odds ratio (OR) = 1.4; 95% confidence interval (CI), 1.0-2.2; p-value for trend = 0.07]. Notably, this association was strongest and statistically significant among children whose mothers reported chemical treatment of the home for pests during pregnancy or childhood (per PON1(-108T) allele: among exposed, OR = 2.6; 95% CI, 1.2-5.5; among unexposed, OR = 0.9; 95% CI, 0.5-1.6) and for primitive neuroectodermal tumors (per PON1(-108T) allele: OR = 2.4; 95% CI, 1.1-5.4). The Q192R polymorphism, which alters the structure of PON1 and influences enzyme activity in a substrate-dependent manner, was not associated with CBT risk, nor was the PON1(C-108T/Q192R) haplotype. These results are consistent with an inverse association between PON1 levels and CBT occurrence, perhaps because of PON1's ability to detoxify OPs common in children's environments. Larger studies that measure plasma PON1 levels and incorporate more accurate estimates of pesticide exposure will be required to confirm these observations

(20) Infante-Rivard C, Labuda D, Krajinovic M, Sinnett D. Risk of childhood leukemia associated with exposure to pesticides and with gene polymorphisms. *Epidemiology.* 1999;10:481-487.
Abstract: We conducted a population-based case-control study of childhood acute lymphoblastic leukemia (ALL) to evaluate the risk posed by reported exposure to pesticides used in and around the home. We compared 491 cases 0-9 years of age to as many controls. We also conducted a case-only study on a subsample of 123 cases to evaluate gene-environment interaction between child genotype and maternal exposure during pregnancy as well as child exposure after birth. We used the polymerase chain reaction (PCR) approach to analyze polymorphisms in CYP1A1, CYP2D6, GSTT1, and GSTM1 genes, which encode enzymes involved in carcinogen metabolism. Indoor use of some insecticides by the owners and pesticide use in the garden and on interior plants, in particular frequent prenatal use, was associated with increased risks up to severalfold in magnitude. Interaction odds ratios were increased among carriers of the CYP1A1m1 and CYP1a1m2 mutations when mother during pregnancy or the child had been exposed to certain indoor insecticides. No such effects were observed in the presence of other tested polymorphisms

(21) Swedish National Chemicals Inspectorate. Human Health Risk Assessment. Proposals for the use of assessment (uncertainty) factors. Application to risk assessment for plant protection products, industrial chemicals and biocidal products within the European Union. KemI Report 1/03. Body for Competence and Methodology Development, National Chemicals Inspectorate and Institute of Environmental Medicine,Karolinska InstitutetSolna, Sweden . 2003.
www.kemi.se/upload/Trycksaker/Pdf/Rapporter/Rapport1_03.pdf
Abstract: This project was conducted within the framework of KOMET, the Swedish National Chemicals Inspectorate (KemI) body for Competence and Methodology Development. The intention of the project was to develop proposals for the use of assessment factors in the human health risk assessment of plant protection products, new and existing industrial chemicals and biocidal products. Another important aim was to provide support and guidance for staff of KemI in their risk assessment work within the EU as well as in all other national and international work.
The work was carried out by KemI in collaboration with the Institute of Environmental Medicine on a consultant basis (IMM, Karolinska Institutet, Solna, Sweden). The project group included Maria Wallén (project leader), Helena Casabona, Claes Debourg, Gregory Moore, and Lena Rosén from KemI, and Agneta Falk Filipsson, Annika Hanberg, and Katarina Victorin from IMM. The project was administrated by Sten-Åke Svensson (KemI) and in the early phase was directed by Malik Altahir (KemI). In addition, Sven Eric Dahlén, Magnus Ingelman Sundberg, Marie Vahter, and Margareta Warholm, all from IMM, and Carola Lidén from the Department of Medicine, Karolinska Institutet and Stockholm County Council, Stockholm, Sweden, contributed to specific sections in Section 5.2.7

"Interindividual variations in sensitivity". The Annexes were prepared by Agneta Falk Filipsson, IMM ("The benchmark dose method") and by Rodolfo Avila, Facilia AB, Bromma ("Probabilistic methods for assessment of health risk of chemicals").

(22) Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: an overview and some results of the italian multicenter case-control study on hematolymphopoietic malignancies. *Ann N Y Acad Sci.* 2006;1076:366-77.:366-377.
Abstract: Exposure to pesticides is recognized as an important environmental risk factor associated with development of cancer. Epidemiological studies, although sometimes contradictory, have linked phenoxy acid herbicides with non-Hodgkin's lymphoma (NHL) and Soft Tissue Sarcoma (STS); organochlorine insecticides with STS, NHL, and leukemia; organophosphorous compounds with NHL and leukemia; and triazine herbicides with ovarian cancer. Exposure assessment is a crucial point in studying the association between cancer and pesticides. In order to investigate the association between hematolymphopoietic malignancies and occupational exposures, including pesticides, a population-based case-control study was carried out in Italy in 11 areas, 9 of which are agricultural or mixed areas. All newly diagnosed cases of hematolymphopoietic malignancies were collected in a 3-year period (1991-1993). The control group consisted of a random sample of the population residing in each area. The approach to infer exposures in agriculture was based on: the use of an agricultural questionnaire with 24 crop-specific questionnaires; expert agronomists who reviewed the collected information for each subject and translated it into pesticides histories. In total, 1925 cases and 1232 controls were interviewed in the nine agricultural areas. Increased risk was observed for some specific classes of pesticides. Furthermore, a nonstatistically significant increased risk of NHL was observed for subjects who were exposed to phenoxy herbicides not using protective equipment and a significant increased risk for exposure to 2, 4-dichlorophenoxy acetic acid (2,4-D)

(23) Mills PK, Yang R, Riordan D. Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988-2001. *Cancer Causes Control.* 2005;16:823-830.
Abstract: OBJECTIVE: Agricultural risk factors for lymphohematopoietic cancers (LHC) in Hispanic farm workers in California were examined in a nested case-control study embedded in a cohort of 139,000 ever members of a farm worker labor union in California. METHODS: Crop and pesticide exposures were estimated by linking county/month and crop specific job history information from union records with California Department of Pesticide Regulation pesticide use reports during the 20-year period prior to cancer diagnosis. RESULTS: A total of 131 LHC diagnosed in California between 1988 and 2001 were included in the analysis. Analyses were conducted by gender and subtype of non-Hodgkins lymphoma (nodal, extra nodal) and by leukemia histology (lymphocytic, granulocytic). Odds ratios were calculated by stratification and by unconditional logistic regression. Risk for all LHC was elevated in workers cultivating vegetables (OR = 1.67, 95% CI = 1.12-2.48). Risk of leukemia was associated with exposure to the pesticides mancozeb (OR = 2.35, 95% CI = 1.12-4.95) and toxaphene (OR = 2.20, 95% CI = 1.04-4.65) while NHL risk was increased in association with 2,4-D (OR = 3.80, 95% CI=1.85-7.81). Risk of leukemia was particularly elevated among female workers and for granulocytic versus lymphocytic leukemia for several chemicals. No associations were noted for multiple myeloma. CONCLUSIONS: California farm workers employed where mancozeb and toxaphene were used had an increased risk of leukemia compared to farm workers employed elsewhere. Employment in farms using 2,4-D was associated with an increased risk of NHL

(24) Mills PK, Yang RC. Agricultural exposures and gastric cancer risk in Hispanic farm workers in California. *Environ Res.* 2007;104:282-289.
Abstract: Previous studies have indicated that farm workers may be at increased risk of gastric cancer. Meta-analyses, ecological, case-control, and cohort studies suggest that some aspects of the agricultural environment may be implicated in the elevated risk. Hispanic farm workers in California are exposed to a multitude of potentially toxic substances in the work site, including excessive sunlight, fertilizers, diesel fumes, and pesticides. A previous analysis of a cohort of California farm workers who had been members of a farm labor union, the United Farm Workers of America (UFW) found a proportionate

cancer incidence ratio for stomach cancer of 1.69 when using the California Hispanic population as the standard. The aim of the current study was to further evaluate associations between gastric cancer and the types of crops and commodities UFW members cultivate and the associated pesticide use as recorded by the California Department of Pesticide Regulation (DPR). We conducted a nested case-control study of gastric cancer embedded in the UFW cohort and identified 100 cases of newly diagnosed gastric cancer between 1988 and 2003. We identified 210 control participants matched on age, gender, ethnicity, and who were known to be alive and resident in California up to the date of the cases' diagnosis. Both stratified analyses and unconditional logistic regression were used to calculate adjusted odds ratios (OR) and 95% confidence intervals (95% CI). Work in the citrus industry was associated with increased gastric cancer (OR=2.88; 95% CI=1.02-8.12) although no other specific crops or commodities were associated with this disease. Working in areas with high use of the phenoxyacetic acid herbicide 2,4-D was associated with gastric cancer (OR=1.85; 95% CI=1.05-3.25); use of the organochlorine insecticide chlordane was also associated with the disease (OR=2.96; 95% CI=1.48-5.94). Gastric cancer was associated with use of the acaricide propargite and the herbicide triflurin (OR=2.86; 95% CI=1.56-5.23 and 1.69, 95% CI=0.99-2.89, respectively). Gastric cancer in California Hispanic farm workers is associated with work in the citrus fruit industry and among those who work in fields treated with 2,4-D, chlordane, propargite, and trifluin. These findings may have larger public health implications especially in those areas of the country where these pesticides are heavily used and where they may be found in the ambient atmosphere

(25) Swan SH, Kruse RL, Liu F et al. Semen quality in relation to biomarkers of pesticide exposure. *Environ Health Perspect.* 2003;111:1478-1484.
Abstract: We previously reported reduced sperm concentration and motility in fertile men in a U.S. agrarian area (Columbia, MO) relative to men from U.S. urban centers (Minneapolis, MN; Los Angeles, CA; New York, NY). In the present study we address the hypothesis that pesticides currently used in agriculture in the Midwest contributed to these differences in semen quality. We selected men in whom all semen parameters (concentration, percentage sperm with normal morphology, and percentage motile sperm) were low (cases) and men in whom all semen parameters were within normal limits (controls) within Missouri and Minnesota (sample sizes of 50 and 36, respectively) and measured metabolites of eight current-use pesticides in urine samples provided at the time of semen collection. All pesticide analyses were conducted blind with respect to center and case-control status. Pesticide metabolite levels were elevated in Missouri cases, compared with controls, for the herbicides alachlor and atrazine and for the insecticide diazinon [2-isopropoxy-4-methyl-pyrimidinol (IMPY)]; for Wilcoxon rank test, p = 0.0007, 0.012, and 0.0004 for alachlor, atrazine, and IMPY, respectively. Men from Missouri with high levels of alachlor or IMPY were significantly more likely to be cases than were men with low levels [odds ratios (ORs) = 30.0 and 16.7 for alachlor and IMPY, respectively], as were men with atrazine levels higher than the limit of detection (OR = 11.3). The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and metolachlor were also associated with poor semen quality in some analyses, whereas acetochlor levels were lower in cases than in controls (p = 0.04). No significant associations were seen for any pesticides within Minnesota, where levels of agricultural pesticides were low, or for the insect repellent DEET (N,N-diethyl-m-toluamide) or the malathion metabolite malathion dicarboxylic acid. These associations between current-use pesticides and reduced semen quality suggest that agricultural chemicals may have contributed to the reduction in semen quality in fertile men from mid-Missouri we reported previously

(26) McDuffie HH, Klaassen DJ, Dosman JA. Is pesticide use related to the risk of primary lung cancer in Saskatchewan? *J Occup Med.* 1990;32:996-1002.
Abstract: Ascertaining cases from a population-based tumor registry in which registration of primary lung cancer is virtually complete, we have consistently found that 40% to 50% of male lung cancer patients were farmers. We interviewed 273 newly diagnosed men and compared their occupational exposures, medical history and smoking characteristics to those of 187 male randomly selected community control subjects. We found that more of the control subjects were farmers (53.5% v 41.4%), that the control subjects tended to have larger farms (P less than .05), and that more control subjects

spilled chemicals on their hands or clothing (47% v 28%, P less than .01) and had an accidental inhalation of a chemical directly into the lung (54% v 33%, P less than .005). We report an absence of correlation of lung cancer risk with occupational exposure to any specific pesticide or pesticides grouped by chemical composition. Adjusting for smoking pack-years or extent of pesticide use did not alter our preadjustment conclusions

(27) McDuffie HH, Pahwa P, McLaughlin JR et al. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev.* 2001;10:1155-1163.
Abstract: Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06-1.81] and to dicamba (OR, 1.88; 95% CI, 1.32-2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22-3.04) and to organophosphorus insecticides (OR, 1.73; 95% CI, 1.27-2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% CI, 1.19-5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CI, 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81); to the insecticides malathion (OR, 1.83; 95% CI, 1.31-2.55), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), carbaryl (OR, 2.11; 95% CI, 1.21-3.69), aldrin, and lindane; and to the fungicides captan and sulfur compounds. In additional multivariate models, which included exposure to other major chemical classes or individual pesticides, personal antecedent cancer, a history of cancer among first-degree relatives, and exposure to mixtures containing dicamba (OR, 1.96; 95% CI, 1.40-2.75) or to mecoprop (OR, 2.22; 95% CI, 1.49-3.29) and to aldrin (OR, 3.42; 95% CI, 1.18-9.95) were significant independent predictors of an increased risk for NHL, whereas a personal history of measles and of allergy desensitization treatments lowered the risk. We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors

(28) McDuffie HH, Pahwa P, Robson D et al. Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. *J Occup Environ Med.* 2005;47:806-816.
Abstract: OBJECTIVE: We sought to test a hypothetical explanation of contradictory results in studies of phenoxyherbicides and NHL, that the exposure of rubber gloves recommended for use by farmers when mixing or applying pesticides simultaneously to 2,4-D (2,4-dichlorophenoxyacetic acid), DEET (N,N-diethyl-m-toluamide), and ultraviolet rays increased their permeability to 2,4-D. METHODS: We conducted a case (NHL n = 513)/control (n = 1506) study among men using age; province of residence; exposure to insect repellents containing DEET, phenoxy-herbicides, or dicamba; and gloves when handling pesticides. RESULTS: Using conditional logistic regression, the stratum with reported exposure to mecoprop, to DEET and the use of rubber gloves had higher odds ratios (3.86; 95% confidence interval = 1.57-9.49) compared with strata with other combinations. CONCLUSIONS: In conclusion, the etiologic complexity of NHL was demonstrated

(29) Pahwa P, McDuffie HH, Dosman JA et al. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. *J Occup Environ Med.* 2006;48:264-274.
Abstract: OBJECTIVE: The objective of this study was to determine if there is an additional risk of developing Hodgkin lymphoma, multiple myeloma, or soft tissue sarcoma as a consequence of exposure

to a combination of phenoxyherbicides, rubber gloves, DEET (N, N-diethyl-m-toluamide), and sunlight compared with each of the individual chemicals. METHODS: This was a population-based study of men with specific cancers and age, province-matched control subjects. RESULTS: No additional risk from these combinations of exposures of developing these three types of tumor was found in contrast to non-Hodgkin lymphoma. CONCLUSIONS: The mechanisms by which phenoxyherbicides contribute to the risk of multiple myeloma and non-Hodgkin lymphoma may be different

(30) Van Maele-Fabry G, Duhayon S, Mertens C, Lison D. Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. *Environ Res.* 2008;106:121-137.
Abstract: PURPOSE: The purpose of this paper is to review available cohort studies and to estimate quantitatively the association between occupational exposure in plants manufacturing pesticides and leukaemia. METHODS: Following a systematic literature search, relative risks were extracted from 14 studies published between 1984 and 2004. Fixed effect analyses were carried out as heterogeneity between studies was not detected. Meta-analyses were performed on the whole set of data and separate analyses were conducted for specific chemical classes of pesticides as well as type of leukaemia. RESULTS: The meta-rate ratio estimate for all studies was 1.43 (95% confidence interval [CI] 1.05-1.94). After stratification by chemical class, consistent increases in the risk of leukaemia were found in all groups but statistical significance was found only for phenoxy herbicides unlikely to have been contaminated with dioxins and furans. This last finding appears equivocal in view of the existing literature. The separate analysis conducted on leukaemias from the myeloid lineage showed the highest relative risk (6.99; 95% CI 1.96-24.90). There was no obvious indication of publication bias. CONCLUSION: The overall meta-analysis among pesticide manufacturing workers provides quantitative evidence to consider occupational exposure to pesticides as a possible risk factor for leukaemia but available data are too scarce for causality ascertainment. Epidemiological evidence did not allow identifying a specific pesticide or chemical class that would be responsible for the increased risk. Exposure to pesticides may be a significant risk factor for specifically developing myeloid leukaemia and there is a need for additional large well-conducted studies with clear definition of exposure and of leukaemia type(s)

(31) Vajdic CM, Fritschi L, Grulich AE et al. Atopy, exposure to pesticides and risk of non-Hodgkin lymphoma. *Int J Cancer.* 2007;.
Abstract: Pesticide exposure has been associated with non-Hodgkin lymphoma (NHL) risk in a number of studies, and two recent studies suggest that the increased risk may be confined to those with a history of asthma. We examined the interaction between occupational pesticide exposure and atopy on risk of NHL in an Australian population-based case-control study. Incident cases (n = 694) were diagnosed in New South Wales or the Australian Capital Territory between 2000 and 2001 and controls (n = 694) were randomly selected from electoral rolls and frequency-matched to cases by age, sex and State of residence. Occupational pesticide exposure was determined by an expert occupational hygienist's assessment of job-specific questionnaires administered by telephone. History of atopy (asthma, hay fever, eczema and food allergy) was self-reported. Logistic regression models included the three matching variables, ethnicity and sun exposure. The OR for NHL with substantial pesticide exposure and any history of asthma was 3.07 (95% CI 0.55-17.10) and with substantial pesticide exposure and no asthma history it was 4.23 (95% CI 1.76-10.16). The p-value for interaction was 0.29. A similar pattern of risk was observed for each of the pesticide subtypes; for asthma at various times of life; for hay fever, eczema, food allergy and any atopy, in men only and for follicular lymphomas only. Although this study had limited power, the findings do not suggest modification of the association between pesticide exposure and NHL risk by asthma or atopic disease more generally. (c) 2007 Wiley-Liss, Inc

(32) De Roos AJ, Gold LS, Wang S et al. Metabolic gene variants and risk of non-Hodgkin's lymphoma. *Cancer Epidemiol Biomarkers Prev.* 2006;15:1647-1653.
Abstract: Genes involved in metabolism of environmental chemical exposures exhibit sequence variability that may mediate the risk of non-Hodgkin's lymphoma. We evaluated associations between non-Hodgkin's lymphoma and 15 variants in AHR, CYP1A1, CYP1A2, CYP1B1, CYP2C9, CYP2E1,

GSTP1, GSTM3, EPHX1, NQO1, and PON1. Cases were identified from four Surveillance, Epidemiology, and End Results registries in the United States, and population-based controls were identified through random-digit dialing and Medicare eligibility files. Metabolic gene variants were characterized for the 1,172 (89% of total) cases and 982 (93%) controls who provided biological samples for genotyping. Subjects who were heterozygous or homozygous for the cytochrome P450 gene variant CYP1B1 V432L G allele were at slightly greater risk of non-Hodgkin's lymphoma [odds ratio (OR), 1.27; 95% confidence interval (95% CI), 0.97-1.65]; these results were consistent across B-cell lymphoma subtypes and among both non-Hispanic White and Black subjects, although not statistically significant. The CYP2E1 -1054T allele was associated with decreased risk of non-Hodgkin's lymphoma (CT and TT genotypes combined OR, 0.59; 95% CI, 0.37-0.93), and this pattern was observed among all histologic subtypes. The numbers of cases of particular subtypes were rather small for stable estimates, but we noted that the PON1 L55M AA allele, associated with slightly increased risk of non-Hodgkin's lymphoma (variant homozygotes OR, 1.36; 95% CI, 0.96-1.95), was most strongly associated with follicular non-Hodgkin's lymphoma and T-cell lymphoma, with ORs for variant homozygotes of 2.12 and 2.93, respectively. There was no overall association with non-Hodgkin's lymphoma for the other gene variants we examined. The modest effects we observed may reflect the context of exposures within the general population represented in our study

(33) De Roos AJ, Zahm SH, Cantor KP et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med.* 2003;60:E11.
Abstract: BACKGROUND: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult. METHODS: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable. RESULTS: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers. CONCLUSION: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios

(34) Infante-Rivard C, Weichenthal S. Pesticides and childhood cancer: an update of Zahm and Ward's 1998 review. *J Toxicol Environ Health B Crit Rev.* 2007;10:81-99.
Abstract: Children are exposed to pesticides through a number of sources, including residential and agricultural applications. Parental occupational exposure to pesticides is also a concern because exposures occurring during pregnancy and carry-home residues also contribute to children's cumulative burden. A number of epidemiological studies consistently reported increased risks between pesticide exposures and childhood leukemia, brain cancer, neuroblastoma, non-Hodgkin's lymphoma, Wilms' tumor, and Ewing's sarcoma. An extensive review of these studies was published in 1998 (Zahm & Ward, 1998). Fifteen case-control studies, 4 cohort studies, and 2 ecological studies have been published since this review, and 15 of these 21 studies reported statistically significant increased risks between either childhood pesticide exposure or parental occupational exposure and childhood cancer. Therefore, one can confidently state that there is at least some association between pesticide exposure and childhood cancer. However, an unambiguous mechanistic cause-and-effect relationship between pesticide exposure and childhood cancer was not demonstrated in these studies, and modifying factors such as genetic predisposition, rarely considered in the reviewed studies, likely play an important role. While the time window of exposure may be a crucial determinant for biological effects associated with pesticide exposure on children, studies have not contributed definitive information on the most vulnerable period. Accurate exposure assessment remains a challenge; future epidemiological studies need to assess gene-environment interactions and use improved exposure measures, including separate

parental interviews, specific pesticide exposure questions, and semiquantitative exposure measures that can be used to confirm information obtained through questionnaires

(35) Bassil KL, Vakil C, Sanborn M, Cole DC, Kaur JS, Kerr KJ. Cancer health effects of pesticides: systematic review. *Can Fam Physician.* 2007;53:1704-1711.
Abstract: OBJECTIVE: To review literature documenting associations between pesticide use and cancer. DATA SOURCES: We searched MEDLINE, PreMedline, CancerLit, and LILACS to find studies published between 1992 and 2003 on non-Hodgkin lymphoma, leukemia, and 8 solid-tumour cancers: brain, breast, kidney, lung, ovarian, pancreatic, prostate, and stomach cancer. STUDY SELECTION: Each title and abstract was assessed for relevance; disagreements among reviewers were resolved by consensus. Studies were assessed by a team of 2 trained reviewers and rated based on methodologic quality according to a 5-page assessment tool and a global assessment scale. Studies rated below a global score of 4 out of 7 were excluded. SYNTHESIS: Most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure. Some showed dose-response relationships, and a few were able to identify specific pesticides. Children's and pregnant women's exposure to pesticides was positively associated with the cancers studied in some studies, as was parents' exposure to pesticides at work. Many studies showed positive associations between pesticide exposure and solid tumours. The most consistent associations were found for brain and prostate cancer. An association was also found between kidney cancer in children and their parents' exposure to pesticides at work. These associations were most consistent for high and prolonged exposures. Specific weaknesses and inherent limitations in epidemiologic studies were noted, particularly around ascertaining whether and how much exposure had taken place. CONCLUSION: Our findings support attempts to reduce exposure to pesticides. Reductions are likely best achieved through decreasing pesticide use for cosmetic (non-commercial) purposes (where children might be exposed) and on the job

(36) Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. Reported residential pesticide use and breast cancer risk on Long Island, New York. *Am J Epidemiol.* 2007;165:643-651.
Abstract: Pesticides, common environmental exposures, have been examined in relation to breast cancer primarily in occupational studies or exposure biomarker studies. No known studies have focused on self-reported residential pesticide use. The authors investigated the association between reported lifetime residential pesticide use and breast cancer risk among women living on Long Island, New York. They conducted a population-based case-control study of 1,508 women newly diagnosed with breast cancer between August 1996 and July 1997 and 1,556 randomly selected, age-frequency-matched controls. Comprehensive residential pesticide use and other risk factors were assessed by using an in-person, interviewer-administered questionnaire. Unconditional logistic regression was used to calculate odds ratios and 95% confidence intervals. Breast cancer risk was associated with ever lifetime residential pesticide use (odds ratio = 1.39, 95% confidence interval: 1.15, 1.68). However, there was no evidence of increasing risk with increasing lifetime applications. Lawn and garden pesticide use was associated with breast cancer risk, but there was no dose response. Little or no association was found for nuisance-pest pesticides, insect repellents, or products to control lice or fleas and ticks on pets. This study is the first known to suggest that self-reported use of residential pesticides may increase breast cancer risk. Further investigation in other populations is necessary to confirm these findings

(37) Sanborn M, Kerr KJ, Sanin LH, Cole DC, Bassil KL, Vakil C. Non-cancer health effects of pesticides. Systematic review and implications for family doctors. *Can Fam Phys.* 2007;53:1712-1720.
Abstract: OBJECTIVE To investigate whether there are associations between exposure to pesticides and 4 chronic noncancer health effects: dermatologic, neurologic, reproductive, and genotoxic effects. DATA SOURCES We searched PreMedline, MEDLINE, and LILACS using the key word pesticide combined with the term for the specific health effect being searched. Reviewers scanned the references of all articles for additional relevant studies.
STUDY SELECTION Studies since 1992 were assessed using structured inclusion and quality-of-methods criteria. Studies scoring <4 on a 7-point global methodologic quality scale were excluded. In total, 124 studies were included. These studies had a mean quality score of 4.88 out of 7.

SYNTHESIS Strong evidence of association with pesticide exposure was found for all neurologic outcomes, genotoxicity, and 4 of 6 reproductive effects: birth defects, fetal death, altered growth, and other outcomes. Exposure to pesticides generally doubled the level of genetic damage as measured by chromosome aberrations in lymphocytes. Only a few high-quality studies focused on the dermatologic effects of pesticides. In some of these studies, rates of dermatitis were higher among those who had had high exposure to pesticides on the job.
CONCLUSION Evidence from research on humans consistently points to positive associations between pesticide exposure and 3 of the 4 non-cancer health outcomes studied. Physicians have a dual role in educating individual patients about the risks of exposure and in reducing exposure in the community by advocating for restrictions on use of pesticides.

(38) Xie L, Thrippleton K, Irwin MA et al. Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci.* 2005;87:391-398.
Abstract: Estrogenic potencies of four herbicides (triclopyr, 2,4-dichlorophenoxyacetic acid (2,4-D), diquat dibromide, glyphosate), two alkylphenol ethoxylate-containing surfactants (R-11 and Target Prospreader Activator (TPA)), and the binary mixture of surfactants with the herbicides were evaluated using an in vivo rainbow trout vitellogenin assay. Juvenile rainbow trout exposed to 2,4-D (1.64 mg/l) for 7 days had a 93-fold increase in plasma vitellogenin (Vtg) levels compared with untreated fish, while rainbow trout exposed to other pesticides alone did not show elevated vitellogenin levels compared to the control fish. When combined with surfactants, trends indicated enhanced estrogenicity for all combinations, but only 2,4-D and triclopyr caused significant induction of Vtg. Concentration-response studies demonstrated that the lowest observed effect concentrations (LOECs) for 2,4-D and triclopyr were 0.164 mg/l and 1 mg/l, respectively. In terms of measured 4-nonylphenol (4-NP), the LOECs of R-11 and TPA were 20 micro/l and 9.5 microg/l, respectively. Binary mixtures of TPA and 2,4-D showed a greater than additive estrogenic response at the lowest concentrations tested, but a less than additive response at the highest combined concentrations. Binary mixtures of TPA with triclopyr also caused greater than additive Vtg responses in two middle concentrations when compared to TPA or triclopyr alone. When trout were exposed to water collected from a site where triclopyr was used in combination with TPA, a concentration-dependent increase in Vtg expression was observed. Measured values of 4-NP were 3.7 microg/l, and triclopyr concentrations were below detection (<5 ng/l). Estradiol equivalents (EEQs) of the lake water were calculated from an estradiol concentration-response curve and were similar (8.5 +/- 7.7 ng/l) to the mean values for the combined triclopyr + TPA treatments (9.9-12.2 ng/l) in the laboratory, suggesting the estrogenicity of the water may have been due to the treatment. These results demonstrated the binary mixture of alkylphenol ethoxylate-containing surfactants with two aquatic pesticides possessed greater than additive estrogenic responses in fish under laboratory conditions and in a field setting

(39) Kim HJ, Park YI, Dong MS. Effects of 2,4-D and DCP on the DHT-induced androgenic action in human prostate cancer cells. *Toxicol Sci.* 2005;88:52-59.
Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) and its metabolite 2,4-dichlorophenol (DCP) are used extensively in agriculture as herbicides, and are suspected of potential endocrine disruptor activity. In a previous study, we showed that these compounds exhibited synergistic androgenic effects by co-treatment with testosterone in the Hershberger assay. To elucidate the mechanisms of the synergistic effects of these compounds on the androgenicity of testosterone, the androgenic action of 2,4-D and DCP was characterized using a mammalian detection system in prostate cancer cell lines. In in vitro assay systems, while 2,4-D or DCP alone did not show androgenic activity, 2,4-D or DCP with 5alpha-dihydroxytestosterone (DHT) exhibited synergistic androgenic activities. Co-treatment of 10 nM 2,4-D or DCP with 10 nM DHT was shown to stimulate the cell proliferation by 1.6-fold, compared to 10 nM DHT alone. In addition, in transient transfection assays, androgen-induced transactivation was also increased to a maximum of 32-fold or 1.28-fold by co-treatment of 2,4-D or DCP with DHT, respectively. However, 2,4-D and DCP exerted no effects on either mRNA or protein levels of AR. In a competitive AR binding assay, 2,4-D and DCP inhibited androgen binding to AR, up to 50% at concentrations of approximately 0.5 microM for both compounds. The nuclear translocation of green

fluorescent protein-AR fusion protein in the presence of DHT was promoted as the result of the addition of 2,4-D and DCP. Collectively, these results that 2,4-D and DCP enhanced DHT-induced AR transcriptional activity might be attributable, at least in part, to the promotion of AR nuclear translocation

(40) Boddington MJ, Gilman AP, Newhook RC, Braun DM, Hay DJ, and Shantora V. Canadian Environmental Protection Act. Priority Substances List Assessment Report No. 1: Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans. Ottawa:Minister of Supply and Services Canada . 1990. http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/dioxins_furans_dioxines_furannes/index_e.html

(41) Holsapple MP, Dooley RK, McNerney PJ, McCay JA. Direct suppression of antibody responses by chlorinated dibenzodioxins in cultured spleen cells from (C57BL/6 x C3H)F1 and DBA/2 mice. *Immunopharmacology.* 1986;12:175-186.
Abstract: Direct addition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 5-20 nM) to cultures of spleen cells from (C57BL/6 x C3H)F1 (B6C3F1) mice produced a suppression of the number of antibody-producing cells which developed in response to lipopolysaccharide, dinitrophenyl-Ficoll and sheep erythrocytes. The suppression of all three parameters was dose-related and parallel. This parallelism and the observation that the magnitude of the suppression was comparable in all three models suggested that the B-lymphocyte was the primary target. The defect was attributed to an effect on early activation or impaired differentiation because direct addition of TCDD had no effect on mitogen-induced proliferation. Temporal studies showed that TCDD produced the greatest suppression of the polyclonal antibody response to lipopolysaccharide when added at the beginning of the culture and that there was no suppression when TCDD was added as soon as 3 h after 200 micrograms/ml lipopolysaccharide. The observation that TCDD could directly suppress the antibody response by spleen cells from DBA/2 mice, at concentrations comparable to those required to suppress the B6C3F1 mice, suggested that the effect on the B-lymphocyte was atypical of the profile of activity (i.e., dependence on the Ah locus) previously reported to characterize the effects of dioxin in other systems. Similar results were demonstrated with congenic mice, as Ahd/d homozygotes were suppressed comparably to Ahb/d heterozygotes. The direct suppression by 2,7-dichlorodibenzo-p-dioxin, a congener previously demonstrated to be devoid of affinity for the Ah locus, further suggests a dissociation from the traditional profile of activity

(42) Holsapple MP, McCay JA, Barnes DW. Immunosuppression without liver induction by subchronic exposure to 2,7-dichlorodibenzo-p-dioxin in adult female B6C3F1 mice. *Toxicol Appl Pharmacol.* 1986;83:445-455.
Abstract: The overall objective of this investigation was to begin to characterize the structure-activity relationship associated with dioxin-induced suppression of humoral immunity. Subchronic exposure (14 days) to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the prototype of the class, produced a suppression of the antibody responses to both sheep erythrocytes, a T-dependent antigen, and dinitrophenyl-Ficoll, a T-independent antigen. Surprisingly, similar results were observed with 2,7-dichlorodibenzo-p-dioxin (DCDD), a dioxin congener lacking affinity for the Ah receptor. In contrast, subchronic exposure to octachlorodibenzo-p-dioxin (OCDD), another dioxin congener without affinity for the Ah receptor, was devoid of activity. Subchronic exposure to 2,3,7,8-TCDD, but not 2,7-DCDD, produced an induction of several liver parameters including: liver weight, amount of microsomal protein, amount of cytochrome P-450, activity of aminopyrine-N-demethylase and activity of aryl hydrocarbon hydroxylase. Subchronic exposure to 2,3,7,8-TCDD or 2,7-DCDD produced no marked changes in thymus weight. Acute exposure to 2,3,7,8-TCDD also produced suppression of the antibody response in the absence of effects on the thymus

(43) Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chlorinated Dibenzo-p-dioxins (CDDs). Agency for Toxic Substances and Disease Registry.USA. 1998. http://www.atsdr.cdc.gov/toxprofiles/tp104.pdf available from http://www.atsdr.cdc.gov/toxprofiles/tp104.html

Pesticide assessment: Protecting public health on the home turf

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M Sears, CR Walker, RHC van der Jagt, P Claman. Pesticide assessment: Protecting public health on the home turf. Paediatr Child Health 2006;11(4):229-234.

Pesticide regulation is examined in the context of Health Canada's Pest Management Regulatory Agency's assessment of the chlorophenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) for turf. 2,4-D is the most common herbicide used to kill weeds in grass.

The medical literature does not uniformly indicate harms from herbicides. However, the balance of epidemiological research suggests that 2,4-D can be persuasively linked to cancers, neurological impairment and reproductive problems. These may arise from 2,4-D itself, from breakdown products or dioxin contamination, or from a combination of chemicals.

Regulators rely largely on toxicology, but experiments may not replicate exposures from 2,4-D application to lawns because environmental breakdown products (eg, 2,4-dichlorophenol) may not accumulate and selected herbicides are possibly less contaminated. Dioxins are bioaccumulative chemicals that may cause cancer, harm neurological development, impair reproduction, disrupt the endocrine system and alter immune function. No dioxin analyses were submitted to the Pest Management Regulatory Agency, and the principal contaminants of 2,4-D are not among the 17 congeners covered in pesticide regulation. Independent assessment of all dioxins is needed, in tissues and in the environment.

The 2,4-D assessment does not approach standards for ethics, rigour or transparency in medical research. Canada needs a stronger regulator for pesticides. Potentially toxic chemicals should not be registered when more benign solutions exist, risks are not clearly quantifiable or potential risks outweigh benefits. Until landscaping pesticides are curtailed nationally, local bylaws and Quebec's Pesticide Code are prudent measures to protect public health. Physicians have a role in public education regarding pesticides.

Key Words: *2,4-dichlorophenoxyacetic acid; Dioxin; Herbicide; Legislation; Pesticide; Toxicity*

L'évaluation des produits antiparasitaires : La protection de la santé publique appliquée aux pelouses

La réglementation des produits antiparasitaires est examinée dans le cadre de l'évaluation, par l'Agence de réglementation de la lutte antiparasitaire de Santé Canada, des utilisations, sur les pelouses, de l'acide 2,4-dichlorophénoxyacétique (2,4-D), un herbicide chlorophénoxy. Le 2,4-D est l'herbicide le plus utilisé pour tuer les mauvaises herbes sur les pelouses. Les publications médicales ne font pas uniformément état des dommages causés par les herbicides. Cependant, selon la majorité des recherches épidémiologiques, le 2,4-D aurait une corrélation étroite avec les cancers, les atteintes neurologiques et les troubles de la reproduction. Cette corrélation peut être attribuable au 2,4-D même, à des produits de dégradation, à la contamination par dioxine ou à une association de produits chimiques.

Les organismes de réglementation se fient largement à la toxicologie, mais les expériences ne répliquent pas nécessairement les expositions à l'application de 2,4-D sur les pelouses, car les produits de dégradation environnementaux (p. ex., 2,4-dichlorophénol) ne s'accumulent peut-être pas et que certains herbicides sont peut-être moins contaminés. Les dioxines sont des produits chimiques bioaccumulatifs qui peuvent être responsables du cancer, porter préjudice au développement neurologique, nuire à la reproduction, perturber le système endocrinien et altérer la fonction immunitaire. Aucune analyse de dioxine n'a été soumise à l'Agence de réglementation de la lutte antiparasitaire, et les principaux contaminants du 2,4-D ne font pas partie des 17 congénères examinés dans la réglementation sur les produits parasitaires. Une évaluation indépendante de toutes les dioxines s'impose, dans les tissus et dans l'environnement.

L'évaluation du 2,4-D ne s'approche pas des normes d'éthique, de rigueur et de transparence imposées par la recherche médicale. Le Canada a besoin d'une réglementation plus ferme à l'égard des produits parasitaires. Les produits chimiques au potentiel toxique ne devraient pas être recensés lorsque des solutions plus inoffensives existent, que les risques ne peuvent être clairement quantifiés ou que les risques potentiels sont supérieurs aux bienfaits. En attendant que les produits parasitaires utilisés pour l'aménagement paysager soient restreints sur la scène nationale, la réglementation locale et le Code de gestion des pesticides du Québec constituent des mesures prudentes pour protéger la santé publique. Les médecins ont une responsabilité dans l'éducation du public à l'égard des produits antiparasitaires.

Pesticides (herbicides, insecticides, fungicides and other '-cides') are spread in the environment for their toxic effects, but does regulation of these high-volume chemicals protect human and ecosystem health? In the present paper, we examine Canada's pesticide regulation in the context of the chlorophenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), the most common herbicide used to kill weeds in grass.

2,4-D, often mixed with other chlorophenoxy herbicides, has been used to kill broadleaf weeds since the 1940s. In 2002 and 2003, commercial lawn care companies in Ottawa applied three metric tons annually of chlorophenoxy herbicide active ingredients (1). 2,4-D is the most common chlorophenoxy herbicide. The United States Environmental Protection Agency (EPA) reports that 66% of 2,4-D is used for agriculture, while 25% of 2,4-D is used for landscaping

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(7% by turf maintenance contractors, 6% by private citizens and 12% in combination products with fertilizers) (2).

Canada's Pest Management Regulatory Agency (PMRA), within Health Canada, registers pesticides for import and sale in Canada. Registration is based on an assessment of risks to human health and the environment, and of efficacy. Importantly, within the legal framework of the Pest Control Products Act (PCPA [3]), benefit is not weighted against risk. The *Proposed Acceptability for Continuing Registration* (PACR) for 2,4-D on turf was published by the PMRA in 2005 (4).

HEALTH ASSESSMENT

The PMRA assesses human health risk chiefly on the basis of animal toxicity studies and human exposure estimates. Many of these studies are proprietary or not peer-reviewed. Moreover, extrapolation from studies of rats may be inappropriate because rats have genes that do not exist in people for the detoxification of chemicals (5), making the setting of 'safety factors' to account for inter- and intraspecies differences somewhat subjective. For instance, in the PACR, a 10-fold safety factor for children's vulnerabilities was frequently reduced to threefold in the modelling of various scenarios.

Exposure estimates are also inexact, especially because 2,4-D is mobile and pervasive. It is washed from lawns into Canadian waterways (6,7) and falls in the rain in the Canadian Prairies (8). It is tracked indoors and, in the absence of degradation by soil microbes and sunlight, it lingers (9). House dust can contribute up to 30% of children's total exposure before application to lawns and up to 76% of the exposure postapplication. The PACR indicates that elimination of 2,4-D from residential landscaping could substantially reduce children's exposure.

In the context of the Declaration of Helsinki (10), epidemiological studies are the chief ethical and publicly available evidence of effects of toxic chemicals on human health. Although many would believe that human trials of pesticide exposure should not be considered by regulators, availability of human test data allows applicants to avoid interspecies 'safety factors' and thereby increase allowable exposures (11). The EPA is proposing to consider intentional human dosing studies for pesticide assessments (12). The PACR cites a report of intentional human dosing with 2,4-D in a slurry with milk (13).

Epidemiology seldom provides absolute proof of harm and cannot discriminate among toxic components in a mixture (2,4-D may be mixed with other herbicides and proprietary 'formulants' to increase tissue penetration and spray performance) or among a variety of exposures (other pesticides, and other occupational or household chemicals). Odds ratios of the harm (eg, cancer) occurring may be decreased to insignificance by many factors (including the 'healthy worker effect'), covariables (such as an active outdoor lifestyle) and reporting mortality rather than incidence while treatments are improving.

The PMRA does not consider many epidemiological studies because it is bound by the PCPA to consider one chemical at a time, and observed epidemiological effects cannot be linked unequivocally to a single chemical. Thus, two separate bodies of evidence are considered by the regulators (animal toxicity and exposure estimates) and the medical community (epidemiology). It may not be a surprise that they reach divergent conclusions regarding the advisability of using 2,4-D on lawns where children play.

With the foregoing caveats, evidence that chlorophenoxy herbicides, including 2,4-D, likely have multiple adverse effects on human health is summarized briefly.

Cancer

The PMRA determined that 2,4-D was not classifiable regarding carcinogenicity, although the International Agency for Research on Cancer classifies 2,4-D as "possibly carcinogenic to humans" (14). The PMRA's independent Science Advisory Panel advised that childhood cancer merited further study, but this was not done because single-agent epidemiological studies are not available. Non-Hodgkin lymphoma, leukemia and sarcoma are frequently noted in association with chlorophenoxy herbicides (15), and the incidence of the intractable childhood cancer neuroblastoma doubles when landscaping pesticides are used around the home (16). These malignancies are increasing in North America and are linked to pesticide exposure (17). The PACR did not reference a 2004 report of a fourfold increase in canine bladder cancer with exposure to chlorophenoxy herbicides (18). The EPA has since revised its guidelines for carcinogenic risk assessment for children, recognizing that children are much more susceptible to carcinogens than adults (19).

Reproductive effects

2,4-D has been found in urine and semen (20), and chlorophenoxy herbicides have been linked to sperm abnormalities (21), increased miscarriage rates (22), difficulties conceiving and bearing children, and birth defects (23). An animal study (24) using an 'off-the-shelf' chlorophenoxy herbicide mixture demonstrated failure of pregnancy. The PACR was published before a reproductive study (required by the PMRA) was received from the Industry Task Force II on 2,4-D Research Data ('industry'). The State of California is now proposing to list 2,4-D products as developmental toxicants under California's Safe Drinking Water and Toxic Enforcement Act (25) based on studies not commonly available (26) and the EPA's recent Reregistration Eligibility Decision for 2,4-D (27).

Neurological impairment

Lawn pesticides are implicated in neurological disorders, such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, autism and attention deficit hyperactivity disorder (15). Mechanisms of neurological harm from pesticides were recently reviewed, including developmental neurotoxicity mechanisms and effects of 2,4-D (28). Possible neurological impairment (dizziness, muscle weakness, loss of coordination and fatigue) is noted on the pesticide label for professional applicators but not for

homeowners. The PACR was published before a developmental neurotoxicity study had been received from industry, although myelin deficiencies were noted in exposed animals in other included studies.

ASSESSMENT DEFICIENCIES

In addition to the required reproductive and neurotoxicity studies noted above that were not in hand at the time of publication of the PACR, contaminants (eg, dioxins), breakdown products (eg, 2,4-dichlorophenol) and product ingredients were not assessed. The quality of evidence and scrutiny required for pesticide registration is much lower than that for a pharmaceutical product, and there is no ongoing surveillance of the extent or effects of cumulative exposures.

Dioxins

Polychlorodibenzodioxins (PCDDs) are formed during chlorophenoxy herbicide manufacturing, with higher-chlorinated congeners and furans being produced at increased temperatures. Normally, dioxins with two or three chlorine atoms are formed in the manufacture of 2,4-D, although higher-chlorinated congeners have been measured in Canadian products (26,29,30). Higher reactor temperatures also favour more rapid and complete conversion of reactants to herbicide during manufacture, so there is a concern that in the absence of enforced regulatory limits, the economic incentive to improve efficiency may foster the production of contaminated herbicides.

PCDDs, furans and polychlorinated biphenyls with four or more chlorine atoms bind with the aryl hydrocarbon receptor (AhR), and thereby trigger a number of toxic effects in mammals (31-33). Outcomes include cancers; endocrine effects, such as diabetes (34); and reproductive problems, such as endometriosis (35), failure to conceive, changed sex ratio of offspring and birth defects. The strength of AhR binding is the basis of dioxin regulation, by which 17 of 76 dibenzo-p-dioxins are regulated (36). It is now recognized, however, that toxic effects of dioxins are also initiated by many other mechanisms (37-41). This throws into question the AhR basis of regulation, particularly because aromatic conjugated ring structures are generally known to possess biological activity and polynuclear aromatic hydrocarbons are known carcinogens (42).

PCDDs with more than two chlorine atoms are 'Track 1 substances', and are targeted for virtual elimination under the Canadian Environmental Protection Act (CEPA) (43). Environment Canada reported that 2,4-D is the second largest chemical source of lower-chlorinated dioxins in Canada (44). Not only was the PACR published before dioxin analyses had been supplied by the industry, the PMRA asked only for analyses of dioxins with four or more chlorine atoms. By focusing on higher-chlorinated congeners, the PMRA is ignoring the bulk of dioxin contamination, as well as the CEPA targets. Prudence calls for independent measurement of all dioxin contaminants – in 'off-the-shelf' products, in areas of highly maintained turf and in biological samples.

Breakdown product – 2,4-dichlorophenol

'Environmental fate' data in the PACR describe the first step in chemical breakdown – disappearance of the parent compound – rather than complete breakdown into basic compounds, such as carbon dioxide and water. The springtime stench in stores and communities without pesticide restrictions is largely the smell of chlorinated phenols. Half-lives reported in the PACR for esters refer to breakdown into the 2,4-D acid, and half-lives for the 2,4-D acid refer to breakdown into 2,4-dichlorophenol. Half-lives for individual parent compounds and breakdown products range up to a month or longer, but exposures resulting from landscaping are considered to be short term (one week) rather than chronic.

Toxicities of breakdown products were not addressed in the PACR. In animals, 2,4-D is excreted largely unchanged; thus, 2,4-dichlorophenol exposures under controlled experimental conditions would be relatively low. However, the United States Centers for Disease Control and Prevention report that 2,4-D degradation is a significant population-wide exposure source for 2,4-dichlorophenol (45). Monitoring indicates that the population is much more heavily contaminated with 2,4-dichlorophenol than with 2,4-D, with urine levels more than one order of magnitude higher. 2,4-dichlorophenol is considered a possible human carcinogen by the International Agency for Research on Cancer (46).

Real products not considered

2,4-D is formulated as a mixture of salts and esters. The diethanolamine salt is particularly toxic and was explicitly excluded from the PACR. However, searches of label information (47), PMRA information requests and fertilizer information provided by the Canadian Food Inspection Agency confirm that 'mixed amines', generally containing diethanolamine salt, are in most herbicide and 'weed and feed'-type products.

2,4-D for lawn care is usually mixed with other pesticides and always with other ingredients. Toxicities of mixtures were not considered, although the aggregate toxicity of all chlorophenoxy herbicides in a mixture should be assessed under the new (2002) PCPA (48). As well, many products containing 2,4-D also contain racemic mecoprop. This chlorophenoxy herbicide is being withdrawn from the market by the manufacturers, who have declined to submit up-to-date data, but it may be sold to homeowners until 2009 (49).

Scientific process

The method for obtaining the highest quality of medical evidence, the randomized controlled trial, is unethical for pesticide testing. However, serious inadequacies in evidence stem from study and review procedures. Pesticide assessment falls short of current best practices by relying on industry-supplied proprietary studies that are not open to independent review and on reviews by interested parties rather than independent systematic reviews of primary literature.

The evidence supporting pesticide registrations is poor compared with that for pharmaceutical trials and federal drug approvals (50), and the drug approval system itself has been found to be lacking. The medical community, through medical journals, has been improving accountability and transparency in pharmaceutical studies by instituting trial registration (51) and standards for trial design, reporting (52) and systematic review (53). Ironically, the PMRA criticized the Ontario College of Family Physicians pesticides report (15) for their lack of inclusion of data (some of which was only available to the PMRA) and for using the well-accepted process of systematic literature review (53).

In response to drug data falsification and withholding of unfavourable information on the part of corporations, the *Journal of the American Medical Association* (JAMA) recently took further steps, requiring independent scrutiny of raw data from trials, with independent researchers accepting scientific responsibility for studies (54). Despite protests (55,56), the JAMA editors insisted, "By virtue of these serious scientific and ethical problems, and the associated lack of trust and lack of confidence they have engendered among physicians and the public, device and drug manufacturers have brought an unprecedented level of 'special scrutiny' on themselves and on the studies they sponsor" (57).

There is considerable corporate overlap between drug and pesticide manufacturers, but no comparable measures of scrutiny are being implemented for pesticides. Furthermore, epidemiological studies are hampered because Canada does not track pesticide sales or use, does not gather information on biological levels of pesticides and other toxic contaminants (58), and has no reporting system for adverse effects (although regulations for a system are under development).

The PMRA was criticized in 2003 by the Office of the Auditor General for failing to re-evaluate older pesticides according to modern standards and for allowing registrations while lacking pertinent information (59). Problems persist with the 2,4-D PACR released in 2005. The new Pest Control Products Act (2002) is not yet in effect but would not have prevented the shortcomings discussed here.

Weighing risks and benefits

The PMRA made an unprecedented declaration of 'safety' (60) on the release of the PACR and the initiation of the public comment period. It seems both inappropriate and possibly dangerous for a regulator to be prejudging the results of missing studies and to be announcing its conclusion before receiving the independent public comment being sought at the time.

Historically, the PMRA has stated that a pesticide "does not pose an unacceptable risk" (61). While science may delineate some elements of risk, the degree of 'acceptability' is an individual choice.

Canadians are realizing that the cumulative effects of myriad ubiquitous synthetic chemicals on humans (especially children), society and ecosystems can never be thoroughly understood. Invoking the precautionary principle, "Where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" (62), many residents are choosing safer, effective strategies for pest control in landscaping. Lower-tier governments are restricting pesticides, including 2,4-D, to protect public health. The Supreme Court of Canada approved Hudson, Quebec's landmark pesticide bylaw in 2001 (Canada's first bylaw) and Toronto's pesticide bylaw in 2005. Bylaws in populous areas (63) and Quebec's Pesticide Management Code (64) have been enacted to protect a growing number of Canadians from landscaping pesticides (over one-third of Canadians at the time of writing). Physicians are speaking out: Toronto Public Health took a lead (65), the Ontario College of Family Physicians systematically reviewed the epidemiology of pesticide harms (15), and the Canadian Medical Association advocated banning combination fertilizer and herbicide ('weed and feed') products (66).

CONCLUSIONS AND RECOMMENDATIONS

Although the medical literature does not uniformly indicate that harms arise from phenoxy herbicide exposure, given the strengths and limitations of epidemiological, toxicological and ecological research, it appears that cancer, neurological impairment and reproductive problems are persuasively linked to phenoxy herbicide exposure. It is not possible to distinguish whether these effects arise from 2,4-D itself, from breakdown products or dioxin contamination, or from a combination of ingredients. However, toxicological experiments using selected (possibly less contaminated) herbicides, and during which typical environmental breakdown products (eg, 2,4-dichlorophenol) would not accumulate, may not be representative of exposures from 2,4-D application to lawns.

Potentially toxic chemicals should not be approved for use when more benign solutions exist, when risks are not clearly quantifiable or when the potential risk outweighs the benefit. In light of what is known and knowable, the use of 2,4-D merely to kill broadleaf weeds on turf is unjustified. Physicians should urge caution in the public debate regarding pesticides for landscaping and point to effective, safer alternative landscaping practices. Organic lawn care focuses on growing thick, healthy turf on rich, aerated soil. Natural products, such as compost, are used to feed the soil and enrich microbial populations that break down thatch, and are pathogens for pests, such as grubs. (This is in contrast to synthetic chemical strategies that eliminate important nontarget organisms, much as antibiotics damage the flora of the gut.) Weeds may be controlled by hand pulling; by using products with ingredients such as corn gluten meal, beet extract or vinegar; and by cutting grass no shorter than 7 cm to shade seedlings. Many companies offer this service, and franchises are now available for entrepreneurs. Canadians are also moving away from monoculture lawns that require a lot of water and energy to turf with a variety of grasses and other species, such as white Dutch clover, for nitrogen fixation and drought resistance, or thyme. Lawns may also be replaced with hardy alternative landscapes, such as native plants.

Until federal legislation curtails nonessential pesticide use nationally, 'cosmetic' pesticide bylaws and provincial legislation, such as Quebec's Pesticide Management Code, are wise, prudent measures to protect public health.

Dioxins are persistent, bioaccumulative chemicals that may cause cancer, harm neurological development, impair reproduction, disrupt the endocrine system and alter immune function. Only 17 of 76 congeners were addressed in the pesticide regulations, and none were analyzed in 2,4-D samples. Dioxins should be monitored comprehensively in people, food and the environment, and phenoxy herbicides should be screened independently for this contamination.

Multiple deficiencies in the 2,4-D PACR illustrate systemic problems with pesticide regulation in Canada. The 2,4-D assessment is contrary to the CEPA and the Declaration of Helsinki, and does not approach standards for ethics, rigour or transparency in medical research. Canada needs a stronger regulator of toxic chemicals, with the competence and will to protect Canadians' health. 2,4-D 'safety' claims should be publicly withdrawn by the PMRA and Health Canada. The 2,4-D PACR should be grounds for federal bureaucratic, legislative and regulatory changes.

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