Comments Submitted to Health Canada’s Pest Management Regulatory Agency

In response to

The Proposed Re-evaluation Decision for Chlorothalonil
(PRVD2011-14, 1 Nov 2011)

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1.0 Introduction

These comments are made in response to the Pest Management Regulatory Agency (PMRA) invitation for public comments on the proposed re-evaluation decision for the fungicide chlorothalonil (Proposed Re-evaluation Decision PRVD2011-14, 1 Nov 2011)\(^1\).

PMRA has proposed to continue to register products containing chlorothalonil for sale and use in Canada, noting that the fungicide is currently registered for a wide range of food crops, on turf (specifically sod farms, and golf greens, tees and fairways), and in paints. The agency acknowledges that Canadians could be exposed to chlorothalonil by consuming food and water, through residential exposure, while working as a mixer/loader/applicator and/or by entering treated sites.

The agency has also stated that hexachlorobenzene, decachlorobiphenyl, pentachlorobenzene, tetrachlorobenzene, chlorinated dioxins and furans expected to be present as contaminants in the chlorothalonil technical grade active ingredients, all of which are classified as Toxic Substances Management Policy Track 1 substances under the Canadian Environmental Protection Act.

Based on observed increases in the incidence of renal adenomas and carcinomas in both sexes of rats and mice that were exposed to chlorothalonil, and an increased incidence of papillomas and/or carcinomas of the forestomach in rats and mice, chlorothalonil has been classified as “likely to be a human carcinogen by all routes of exposure” by the US Environmental Protection Agency (EPA). Nonetheless, PMRA has proposed the registration of chlorothalonil be continued for use in Canada, and doing so, it has proposed new risk reduction measures (to be implemented via label changes) stating that it believes that, with the proposed changes, that chlorothalonil is unlikely to affect the health of Canadians when used according to the label directions. PMRA also has indicated that it believes that that exposure dose levels have been established that protect the most sensitive subgroups within the Canadian population.

Accordingly, these comments are being submitted to PMRA to highlight four significant health-related concerns about the risks that Canadians will face with ongoing exposures to chlorothalonil, and to ask the agency to discontinue the registration of all products containing the chemical, until a number of significant additional evaluation criteria have been established and met.

The main concerns that will be outlined in this document are summarized as follows:

a) That the risk assessment methods that have been used by PMRA to assess the carcinogenic potential of the chemical, are not robust enough to determine safe levels of exposure for the chemical given the proposed conditions of registration. Specifically, PMRA has not assessed the cumulative effects of chlorothalonil when combined with other pest control products that have a common mechanism of toxicity, and therefore that the agency cannot be reasonably certain
that no harm to human health will occur if the registration of the product is continued as proposed.

b) That hexachlorobenzene, decachlorobiphenyl, pentachlorobenzene, tetrachlorobenzene, chlorinated dioxins and furans are all expected to be present in the chlorothalonil technical grade active ingredients, and all are classified as Toxic Substances Management Policy Track 1 contaminants. Canada has longstanding international obligations under the Stockholm Convention to eliminate these substances, and both Heath Canada and Environment Canada have enacted policies to ensure these obligations are met. Yet PMRA has failed to implement ministerial policy and the proposed continuation of the registration of chlorothalonil will allow substantial ongoing releases of these persistent chemicals into the environment. This will result in foreseeable harm to Canadians, which would be in violation of the Pest Control products Act.

c) That PMRA cannot be reasonably certain that no harm to human health will come to the most sensitive major identifiable subgroups within the Canadian population (i.e., Canadians living with cancer, those who are overweight, pregnant women, fetuses exposed in utero and nursing infants, and those who smoke).

d) That PMRA has also not taken into consideration the cumulative effects of chlorothalonil and other PMRA registered chemicals that directly contribute to carcinogenicity by other relevant modes of action. Therefore the agency cannot be reasonably certain that no harm to human health will occur if the registration of the product is continued as proposed.

1.1. Terminology

To clarify the terminology being used in this submission, we highlight the fact the World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) Workshop on Aggregate/Cumulative Risk Assessment (Combined Exposures to Multiple Chemicals) was held in Washington, DC, USA, on 19–21 March 2007. This workshop is important because one of the principal objectives of the activity, which involved experts from agencies worldwide, was to consider the state of the art of the science related to cumulative risk assessment. In a peer-reviewed article that explained the outcomes of that meeting, Bette Meek (McLaughlin Centre, Institute of Population Health, University of Ottawa, Ottawa, Ontario, Canada) and her colleagues explain that chemicals that act by the same “mode of action” often act in a potency-corrected “dose additive” manner (noting that the term “mode of action” has been defined as “a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data”). The concept of “mode of action” is therefore highlighted here as being centrally important in the assessment of cumulative risks from a human health perspective.
In the United States, the EPA’s 2002 Guidance document on cumulative risk notes that a “common mechanism of toxicity” pertains to “two or more pesticide chemicals or other substances that cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events (i.e., interpreted as mode of action)”\(^3\). And the EPA’s 2005 guidance on Carcinogenic Risk Assessment defines *mode of action* as “a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation (where a *key event* is defined as an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element)\(^4\).

Notably, the term “mode of action” is specifically contrasted with *mechanism of action,* which implies a more detailed understanding and description of events (often at the molecular level) than is meant by mode of action. The EPA guidance further explains that there are many examples of possible modes of (carcinogenic) action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression\(^4\).

To be clear, it is important to point out that the EPA has adopted the term “mode of action” for cancer risk assessments and it has developed a definition that is largely consistent with the WHO/IPCS definition for the same term. However, the agency has also chosen to offer descriptive titles for their examples of possible modes of (carcinogenic) action, that are, in fact, the names of “key events” (i.e., empirically observable precursor steps that are necessary elements of the mode of action). For example, the EPA suggests that “inhibition of cell death” is a mode of (carcinogenic) action, but the inhibition of cell death is actually a key event (i.e., it is not the entire sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation).

The same can be said for the other examples that the EPA offers as possible modes of action (i.e., mutagenicity, mitogenesis, cytotoxicity with reparative cell proliferation, and immune suppression\(^4\)). Each of these titles is the name of a key event (i.e., not an entire sequence of steps that results in cancer). At first glance, this appears to be an inconsistent use of terminology used in the labelling the various modes of action. However, the EPA’s choice to use the names of key initiating events as the titles for modes of (carcinogenic) action is intuitive and practical, because each of these terms does describe an important instigating event that either leads to cancer or directly contributes to cancer causation.

While the EPA’s guidance on this topic suggests that there are “many examples of possible modes of carcinogenic action”\(^4\), the science of cancer has advanced considerably in the past decade and a mature organizing framework has emerged that simplifies this nomenclature task considerably. The most complete, most frequently cited, and best recognized model of cancer that captures all of these key events in a holistic and comprehensive manner is the “hallmarks of cancer” framework\(^5,6\).
The original hallmarks of cancer framework was first offered by Hanahan and Weinberg in 2000, and over the past decade it has been refined considerably using inputs, suggestions and an aggregation of new research from around the globe. The latest version of this framework was published in March of 2011, so it represents a state-of-the-art understanding of the disease. The framework is introduced here, because it easily encompasses the modes of (carcinogenic) action proposed by the EPA, and it helps us to quickly organize other key events that are involved in cancer causation. The framework also helps us to understand how these key events are related to one another.

In essence, all cancers are comprised of a grouping of misbehaving (immortalized) cells that are growing, dividing and replicating repeatedly (thereby forming tumours), and while there are many types of cancer, it is now understood that cancers of all types are largely driven by these 10 prominent hallmark characteristics or key events:

1. Genetic Instability
2. Tumor Promoting Inflammation
3. Sustained Proliferative Signalling**
4. Evasion of Anti-growth Signalling**
5. Resistance to Programmed Cell Death (i.e., apoptosis)**
6. Replicative Immortality (bypassing senescence) **
7. Reprogramming of Cellular Energy Metabolism (i.e., mitochondrial shift to glycolysis)
8. Immune System Evasion
9. Angiogenesis **
10. Tissue Invasion and Metastasis**

So, for the sake of this submission, we have adopted a use of terminology that follows the EPA’s approach to the titling each mode of action. Since each of these ten canonical categories describes a high level key event that is known to either (1) instigate, and ultimately cause cancer, or (2) make an important contribution to cancer causation, we will use each of these ten titles as the names for ten modes of (carcinogenic) action that are well supported in the academic literature (i.e., each is known to contribute to the cumulative “sequence of key events and processes” leading to cancer).

Note that the first two modes of (carcinogenic) action (i.e., genetic instability and tumor-promoting inflammation) are considered to be enabling because they do not always cause cancer, but they are frequently seen in most cancers and they can cause cells to transition from a normal to a carcinogenic state.

Six of the remaining eight modes of (carcinogenic) action (i.e., those highlighted above with double asterisks) are widely accepted as being shared attributes found in all cancers.
Finally “Reprogramming of Cellular Energy Metabolism” and “Immune System Evasion” have emerged as two important modes of (carcinogenic) action that contribute to most cancers, but they are not necessarily present in all cancers.

2.0 Obligation to Assess Cumulative Effects

These comments are being submitted because the Minister has an obligation under subparagraph 19(2)(b)(i) of the Pest Control Products Act to consider available information on the cumulative effects of chlorothalonil and other pest control products that have a common mechanism of toxicity. Yet, in this instance, PMRA has relied upon recent EPA risk assessments of the carcinogenicity of chlorothalonil, and that has caused PMRA to miss this very important legal requirement.

Yet it is apparent in the proposed decision document that the agency has not assessed the cumulative effects of chlorothalonil when combined with other pest control products that have a common mechanism of toxicity as required by subparagraph 19(2)(b)(i) of the Pest Control Products Act, and therefore it is suggested here that the agency cannot be reasonably certain that no unacceptable risk of harm to human health, including future generations, will occur if the registration of this chemical is continued as proposed.

In the proposed decision document, PMRA has relied heavily upon the evaluation work that has already been carried out by the US EPA, but the agency has failed to address the fact that all of the risk-assessment calculations that have been employed by the EPA and by PMRA (to determine safe exposure levels for the product) have been based on tests that only assessed the carcinogenic effects of chlorothalonil when tested in isolation. PMRA noted that the EPA has not determined whether chlorothalonil has a common mechanism of toxicity with other pest control products, and explained that it was therefore assumed that chlorothalonil does not share a common mechanism of toxicity with other pest control products, and concluded that a cumulative risk assessment was therefore not required. However, the modes of action by which chlorothalonil exerts its carcinogenicity have been well documented, and there are other pesticides that have been registered and approved for use both in Canada and the United States that have also been shown to exert their carcinogenic potential via the same modes of action. So this appears to be a gross error on the part of the EPA.

The EPA is required by US law to consider cumulative toxicological effects (a requirement that applies to all types of toxicological effects, including cancer), but just because the US agency has not identified any other substances that share common modes of (carcinogenic) action with chlorothalonil, does not relieve the Minister of her obligation to assess the cumulative effects of chlorothalonil when combined with other pest control products that have a common mechanism of toxicity, as this is required by subparagraph 19(2)(b)(i) of the Pest Control Products Act.
If the Minister proposes to continue to allow the registration of chlorothalonil, the agency must assess the cumulative effects of the chemical when it is combined in the environment with other approved pesticides that act on the same modes of action, as these additional influences have the potential to produce additive and/or synergistic effects. Otherwise the risk exposure estimates that PMRA has developed (based only on the effects observed in tests of chlorothalonil alone) may underestimate the potential risks that Canadians face, and the agency will not be able to assess the cumulative impact that these chemicals will have on the incidence of cancer at the population level. And, in that instance, the agency would not be reasonably certain that harm to human health, including future generations, will occur if the registration of the product is continued as proposed, which is an unacceptable level of risk (as defined by the subsection 2 (2) of the Pest Control Products Act).

2.1 Chlorothalonil’s Modes of (Carcinogenic) Action

The most recent review of the carcinogenicity of chlorothalonil was undertaken by the California Environmental Protection Agency, Reproductive and Cancer Hazard Assessment Branch, Office of Environmental Health Hazard Assessment (OEHHA) in March of 2011 (attached as Appendix A). As part of that study, the agency assessed the dose-response relationship for chlorothalonil (as it related to carcinogenicity), and the following modes of (carcinogenic) action were identified:

2.1.1 Genetic Instability

According to the California Environmental Protection Agency, the evidence indicates that chlorothalonil can induce DNA damage (i.e., is genotoxic), and cause genetic instability. The agency states that: “Evidence that chlorothalonil induces DNA damage includes studies showing that it increases levels of the oxidized DNA product 8-hydroxy-2'-deoxyguanosine (8-OH-2dG), which is a mutagenic adduct, in rat liver in vivo in a dose-dependent manner (Lodovici et al., 1997). Additional evidence comes from various in vitro and in vivo studies measuring DNA damage, as detected by the comet assay, in rodents and Humans”.

Furthermore, they add that “In a study of male farmers in which mononuclear leukocytes were evaluated before and after a single day of spraying various mixtures of pesticides, increased DNA damage was observed in farmers that sprayed mixtures containing chlorothalonil (Lebailly et al., 1998). This DNA damage was observed in the absence of cytotoxicity or other effects on hematologic parameters, and was attributed by Lebailly et al. (1998) to chlorothalonil exposure”.

The reviewers also noted that chlorothalonil binds to cellular proteins, including histones in the cell nucleus and pointed out that this could disrupt DNA replication and transcription processes, effect alterations to DNA structure (e.g., folding and packaging), cause DNA strand breaks, and alterations in global gene methylation level with resultant changes in gene expression patterns.

As well, the primary action of chlorothalonil is to deplete glutathione stores in cells as it is converted to reactive intermediates (producing oxygen free radicals in the process). Intracellular glutathione is
depleted because it is being used to inactivate these reactive metabolites thus protecting against cellular damage. However, once depleted this can mean that no remaining defence is possible against excess exposures to chlorothalonil, or to any other chemical that might cause toxicity via reactive intermediate formation. Thus this mechanism of glutathione depletion opens a wide range of possible adverse cumulative interactions with other chemicals that would otherwise also require glutathione detoxification.

All of these aspects of chlorothalonil’s actions are important because genetic instability can drive the process of cancer causation\textsuperscript{11}. Cancer is a disease, with many genes in many diverse pathways that are typically involved in its multistep process of enablement, and it is widely accepted that the accumulation of mutations that promote the immortalization of proliferating cells (i.e., activate oncogenes) and disrupt the mechanisms which should stop this from occurring (i.e, disable tumour suppressor genes)\textsuperscript{12,13} can cause cancer. Genetic instability itself is therefore a mode of (carcinogenic) action that has the potential to promote unscheduled genetic alterations and cause cancer.

So this mode of (carcinogenic) action encompasses a diverse range of genetic changes, including instability occurring at the chromosomal level and at the nucleotide level, and it can involve disrupted DNA repair systems as well, and the cumulative effect that all PMRA registered pesticides have on genetic instability is extremely important. Since genetic damage can be random, and since the genome controls all aspects of cellular function, random genetic damage can potentially enable any of the other modes of action (as illustrated in the schematic diagram below).

\textbf{Chlorothanil (via DNA Damage)}
2.1.2 Sustained Proliferative Signalling

According to the California Environmental Protection Agency, the evidence indicates that chlorothalonil consistently induces cell proliferation via growth signalling. This is supported by empirical observations:

“Chlorothalonil induced cell proliferation in the forestomach and kidneys of male and female rats in long-term bioassays (e.g., Wilson, 1985 and Wilson, 1986, as reported in CDPR, 2005; Wilson and Killeen, 1989; Wilkinson and Killeen, 1996). Chlorothalonil has also been shown in shorter-term studies to induce sustained cell proliferation in rat forestomach and kidneys (U.S. EPA, 1999; Wilkinson and Killeen, 1996). For example, in 90-day dietary studies in male F344 rats, increased BrdU labeling was observed in the kidneys at day 7, day 28, and day 91 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999). Similarly, in 28-day dietary studies in male rats, increased proliferating cell nuclear antigen (PCNA) staining of proximal convoluted tubule epithelial cells and increased BrdU labeling of the forestomach were observed at day 7, day 14, day 21, and day 28 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999).”

The reviewers suggest two possible mechanisms by which chlorothalonil induces cell proliferation. One proposed mechanism involves the induction of cytotoxicity, accompanied by regenerative hyperplasia (a type of cell proliferation). In this regard, they note that “chlorothalonil-derived thiols have been shown to inhibit mitochondrial respiration, based on studies conducted with rat kidney subcellular fractions (Wilkinson and Killeen, 1996). Inhibition of mitochondrial respiration results in decreased formation of adenosine triphosphate (ATP), increased oxidative stress, and ultimately, cell death (Anders and Dekant, 1998). Wilkinson and Killeen (1996) proposed that cytotoxicity induced by chlorothalonil-derived thiols in the kidney leads to compensatory cell proliferation and hyperplasia that, if sustained, eventually results in tumor formation.”

Support for the second possible mechanism by which chlorothalonil induces cell proliferation mechanism comes from studies in LNCaP cells, a human prostate cancer cell line, in which chlorothalonil treatment increased ErbB-2/HER-2 tyrosine kinase activity, mitogen-activated protein kinase (MAPK) phosphorylation, and cell proliferation.

This is quite important, because aberrant activation of the ErbB-2/HER-2 receptor has been the subject of an intense amount of research and it is well-established that the activation of this receptor also promotes cellular resistance to programmed cell death, and tissue invasion and metastasis (as illustrated below).
The research that determined that chlorothalonil instigates the ErbB-2/HER-2 tyrosine kinase signal transduction pathway was conducted by researchers in the Department of Environmental Toxicology and the Center for Environmental Health Sciences at the University of California, Davis. While this is the only study that has shown this relationship, the experiments were all run in triplicate or quadruplicate, and each experiment was repeated 2 or 3 times\(^9\).

Notably, aberrant activation of the HER-2 receptor has been implicated in breast, colon, bladder, ovarian, endometrial, lung, uterine cervix, head and neck, esophageal, and gastric carcinomas\(^{19}\), and it is a well-established pathway in carcinogenesis. Indeed it is so important that it is already the target of anticancer therapeutics such as trastuzumab/Herceptin, a drug that is primarily used in breast cancers (one that exerts its anti-cancer effect by blocking the HER-2 receptor from being activated)\(^{20}\).

### 2.2 Common Modes of (Carcinogenic) Action

To underscore the importance of assessing cumulative exposures to other pesticides that have common mechanisms of toxicity, the following examples of PMRA approved chemicals are provided. Each of these chemicals will be shown to contribute to the same modes of action that have been attributed to chlorothalonil. Also, the impact of not assessing the cumulative effects of these exposures combined with exposures to chlorothalonil will be discussed.
2.2.1 Genetic Instability

One PRMA registered pesticide that acts via this mode of (carcinogenic) action is methomyl. Methomyl is currently used on a range of food crops (PMRA Re-evaluation Note REV2010-08, Methomyl) and, it is noted in PMRA’s Re-evaluation Note (REV2009-02 Preliminary Risk and Value Assessments of Methomyl, 14 January 2009) that methomyl demonstrated mutagenic potential in human lymphocytes in vitro (as indicated by an increase in micronuclei and chromosomal aberrations) and that it caused DNA damage in vivo in the mouse. While it has also been noted that methomyl did not show evidence of carcinogenicity in the mouse or rat, this alone does not mean that the cumulative effects of DNA damage from methomyl and chlorothalonil combined can be dismissed. If cancer can be directly instigated by an accumulation of DNA damage that results in genetic instability, then the cumulative effects of these registered pesticides is important, and this common/shared mode of action must be taken into account where carcinogenicity is a concern.

Similarly, PMRA also lists Paraquat as a registered product (Re-evaluation Decision Document: Paraquat Dichloride RRD2006-13, 29 March 2006). This particular herbicide is used to control many grasses and broad-leaved weeds on a variety of fruit, vegetable, and other field crops, nursery crops, shelterbelts and non-food aquatic sites. The Canadian population is therefore exposed to the chemical in food and water, and can also be exposed when handling the chemical. While Paraquat itself is not considered to be carcinogenic by PMRA, the chemical is considered to be a prototypical redox cycling agent that is commonly used experimentally to cause mitochondrial damage, reactive oxygen species and oxidative stress. Again, this is highly relevant to cancer because in "oxidative stress", reactive oxygen species production has been known to cause DNA damage, and if sufficient damage occurs genetic instability results. In other words, Paraquat’s longstanding status as a well-established inducer of oxidative stress make it a direct contributor to genetic instability. Therefore its cumulative effects when combined with chlorothalonil must also be considered (i.e., given that genetic instability is an important mode of action that has the potential to instigate cancer).

Lastly, and from a slightly different perspective, it needs to be pointed out that PMRA also currently has two registered products containing the chemical ethylenediaminetetraacetic acid or EDTA. These are known as Ferric Sodium EDTA (Registration Decision, RD2008-04, 4 April 2008) and FeHEDTA (Registration Decision RD2010-09, 22 September 2010) that can contribute to genetic instability. Ferric sodium EDTA is a molluscicide used to control slugs and snails in a variety of fruit trees, turf, grasses, vegetables, berries and ornaments in greenhouses and outdoors, whereas FeHEDTA is a selective herbicide used on lawns. Again, Canadians can be exposed to both products when handling and applying the product, and they can be exposed to Ferric sodium EDTA in both water and food. And PMRA’s proposed decision documents for both of these products make it clear that there have been no indications of genotoxicity or carcinogenicity. However, a review of the open literature on EDTA shows that this is a disruptive chemical that inhibits DNA repair.
A review of EDTA in 1983\(^2\) summarized this disruptive ability by pointing out that it had been well established that EDTA influences chromosome breakage by mutagenic agents. In particular, when applied in combination with chemical mutagens, EDTA increases the frequency of mutagen-induced aberrations, by interfering with DNA repair. So while EDTA may be seen as a harmless compound by PMRA, it has been known for almost 30 years that it is disruptive of DNA repair, which makes it a potentially important contributor to genetic instability. Therefore its cumulative effects (i.e., when combined chlorothalonil) must also be considered because an impaired DNA repair capability, increases genetic instability and elevates cancer risks (because unrepaired damage can lead to higher frequencies of mutations which can instigate the other mechanisms that are involved in cancer causation).

The diagram below illustrates that both chlorothalonil and methomyl contribute to genetic instability directly by damaging DNA. While Paraquat acts on this mechanism (i.e., genetic instability) by causing mitochondrial damage which generates reactive oxygen species, and that the EDTA-based pesticides have the potential to exacerbate the genetic instability caused by chlorothalonil, methomyl, paraquat, Ferric Sodium EDTA and FeHEDTA (by disrupting normal DNA repair abilities).

In summary, the Minister has an obligation under subparagraph 19(2)(b)(i) of the Pest Control Products Act to consider available information on the cumulative effects of chlorothalonil and other pest control products that have a common mechanism of toxicity. But it should now be apparent that PMRA has not
adequately considered the cumulative effects of chlorothalonil and other approved pesticides that can exert their carcinogenic potential via the same mode of action (i.e., genetic instability).

As a result, the agency cannot say with reasonable certainty that it knows what will happen when low doses of chlorothalonil (which contributes to genetic instability by inducing DNA damage), low doses of methomyl (which contributes to genetic instability by causing DNA damage), low doses of Paraquat (which also contributes to genetic instability via oxidative stress) are combined with low doses of the EDTA-based products (which contribute to genetic instability by disrupting DNA repair). Yet the combination of all of these chemicals appears to represent just the sort of cumulative effect that has the potential to dramatically increase the risks of genetic instability, which can cause cancer.

Furthermore, these examples of PMRA registered pesticides that can contribute to genetic instability are just a sampling that have been provided to illustrate the potential for increased risk that is associated with cumulative exposures that act via the same mode of action. There are many other PMRA registered pesticides that have also been shown to contribute to genetic instability, but the cumulative effects that these chemicals have on genetic instability have not been considered by the agency.

The Minister therefore cannot be reasonably certain that PMRAs risk estimates are accurate nor can she be certain that PMRA’s proposed margins of safety are adequate. Therefore, she cannot be reasonably certain that no harm to human health will occur if the registration of the product is continued as proposed (which is an unacceptable level of risk as defined by the Pest Control Products Act [2.(2)]).

### 2.2.1.1 Recommendations regarding Genetic Instability

It is recommended that PMRA review all data pertaining to the potential for each of its registered products to contribute to genetic instability, and then factor the cumulative effects of the anticipated exposures from all of these products into its risk assessment of chlorothalonil before proceeding with a decision to continue with the registration of the chemical, as required by the Pest Control Products Act [19. (2) (b)].

### 2.2.2 Sustained Proliferative Signalling

The second mode of action that can be directly attributed to chlorothalonil is that of sustained growth signalling leading to cellular proliferation. Again, this is an area where the agency has not assessed the cumulative effects of chlorothalonil when combined with other pest control products that have a common mechanism of toxicity as required by subparagraph 19(2)(b)(i) of the Pest Control Products Act.

For example, in a recent registration decision, PMRA approved Flonicamid (Registration Decision RD2011-01, 24 January 2011), an insecticide that is used to controls aphids (noting that Canadians face
potential exposure to the chemical through the diet, i.e., food and water). This chemical is used on a wide range of food crops, and Canadians can also be exposed also when handling and applying the product. In PMRA’s Proposed Registration Decision document (PRD2010-25 Flonicamid, 15 October 2010), the agency noted that Flonicamid was capable of exerting a number of negative health effects in animals given daily doses of Flonicamid over long periods of time including effects on the liver, kidney, spleen, bone marrow and lung. From a carcinogenicity perspective, Flonicamid produced a statistically significant increase in alveolar/bronchiolar tumours in mice of both sexes. It was suggested that these tumors were attributed to a mitogenic ability that the chemical has to instigate cellular growth and division, which results in increased cellular proliferation in a certain type of cell (i.e., Clara cells). Microscopic examinations that were subsequently conducted confirmed that these changes did occur in Clara cells. However, these cells are not unique to mice (they also occur in humans), and the possibility that the chemical might have a similar proliferative effects on other cells has not been ruled out. In other words, Flonicamid has the ability to instigate sustained proliferative signalling, which is a mode of carcinogenic action that it shares in common with chlorothalonil.

The diagram below illustrates that both chlorothalonil and Flonicamid are known to cause sustained growth signalling which ultimately results in cellular proliferation. Yet PMRA has not considered the cumulative effects of chlorothalonil when it is combined with Flonicamid. Nor does it appear that the agency has made any attempt to consider the cumulative effects that might be relevant for other PMRA registered pesticides that may have also been shown to induce sustained proliferative signalling.

Again, unless PMRA assesses the cumulative effects that these combined exposures will have on this important mechanism of carcinogenicity, the margins of safety that PMRA has developed will be flawed. Additional chemicals acting via the same mode of action have the potential to act in an additive and/or
synergistic manner. Therefore the agency cannot be reasonably certain that no harm will come to Canadians if they are exposed to chlorothalonil given the proposed conditions of registration.

2.2.2.1 Recommendations regarding Sustained Proliferative Signalling

It is therefore recommended that PMRA ensure they have data pertaining to the potential for each of its registered products to contribute to the sustainment of Proliferative Signalling in cells, and then factor the cumulative effects of the anticipated exposures from all of these products into its risk assessment of chlorothalonil (before proceeding with a decision to continue with the registration of the chemical) as required by the Pest Control Products Act [19. (2) (b)].

2.2.3 Resistance to Programmed Cell Death

The third mode of action that can be attributed to chlorothalonil is that of promoting cellular Resistance to Programmed Cell Death (i.e., apoptosis). No direct evidence of chlorothalonil causing resistance to programmed cell death exists. However, chlorothalonil has been definitively shown to activate the HER-2 tyrosine kinase signal transduction pathway, which confers cellular resistance to apoptosis by activating cell survival pathways. So again, this is an area where the agency has not assessed the cumulative effects of chlorothalonil when combined with other pest control products that have a common mechanism of toxicity as required by the subparagraph 19(2)(b)(i) of the Pest Control Products Act.

For example Malathion is a PMRA registered insecticide and acaricide used to control a broad range of insects and pests at a wide variety of sites in Canada, including food and feed crops, and residential and recreational areas. Potential exposure to Malathion can occur through the diet (food and water) or when handling and applying the product. Malathion is highlighted here because a number of mechanistic studies have shown that Malathion is capable of creating mutations that disrupt the function of the p53 tumor suppressor, a mechanism that is critically important for proper functioning of programmed cell death. Indeed, the p53 tumor suppressor is estimated to be mutated in roughly half of the many different types of cancer.

In PMRA’s recent Proposed Re-evaluation Decision for Malathion, (PRVD2010-18 Malathion, November 2010), the agency noted that Malathion was not found to be genotoxic, and added that it was unlikely to pose a carcinogenic risk for humans. In chronic feeding studies performed with Malathion in mice and rats, treatment-related increases in benign tumor incidences were observed in the liver (mouse, rat) and in the nasal/oral cavity (rat). The EPA classifies Malathion as “suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential”. Nonetheless, PMRA has used a weight of evidence approach to support its contention that Malathion is unlikely to possess carcinogenic potential.
for humans. But it is still important for PMRA to acknowledge the chemical as a potentially disruptive agent that may have the ability to contribute to cellular Resistance to Programmed Cell Death mode of action (via p53 mutation).

The diagram below illustrates that chlorothalonil has the potential to enable resistance to programmed cell death via the HER-2 receptor, and that Malathion also has the ability to confer this ability via mutations in p53. So both chemicals share in their ability to contribute to this very important mode of action, yet PMRA has not considered the cumulative effects of combined exposures to both of these chemicals.

Again, unless PMRA assesses the cumulative effects that these combined exposures will have on this important mode of action, the risk assessments for chlorothalonil, and the margins of safety that have been calculated are unlikely to be accurate. Therefore the agency cannot be reasonably certain that no harm will come to Canadians if the use of chlorothalonil continues given the proposed conditions of registration.

2.2.3.1 Recommendations regarding Resistance to Programmed Cell Death

It is therefore recommended that PMRA ensure they have data pertaining to the potential for each of its registered products to contribute to cellular-level Resistance to Programmed Cell Death, and then ensure that they factor the cumulative effects of the anticipated exposures from all of these products into its risk assessment of chlorothalonil (before proceeding with a decision to continue with the registration of the chemical) as required by the Pest Control Products Act [19. (2) (b)].
2.2.4 Tissue Invasion & Metastasis

The fourth mode of action that can be attributed to chlorothalonil is that of Tissue Invasion and Metastasis. No direct evidence of chlorothalonil causing tissue invasion and metastasis exists. However, as noted above, chlorothalonil has been definitively shown to activate the HER-2 tyrosine kinase signal transduction pathway\(^8,14\), which is a pathway that does enable this important mechanism\(^{17,18}\). So again, this is an area where the agency has not assessed the cumulative effects of chlorothalonil when combined with other pest control products that it has already registered as required by subparagraph 19(2)(b)(i) of the Pest Control Products Act.

In this instance, the herbicide Roundup (containing the technical ingredient glyphosate), and the insecticides Lorsban (containing the technical ingredient chlorpyrifos) and Warrior (containing the technical ingredient lambda-cyhalothrin) are all PMRA registered pesticides that have been shown to have the ability to contribute to tissue invasion and metastasis. Specifically, a 2005 study at the Division of Oncology at the Duke University Medical Center in Durham, North Carolina showed that the herbicide Roundup and insecticides Lorsban and Warrior induced The urokinase plasminogen activator (uPA) and that Lorsban and Warrior also induced uPAR, its receptor\(^{39}\). This is important because this activator and receptor play a major role in adhesion, migration, invasion and metastasis of cancer cells\(^{40,41}\).

The Duke researchers also found that a combination of Roundup and Lorsban or a combination of Roundup and Warrior produced greater increases in uPA and uPAR than when the agents were used alone, which underscores the importance of assessing the cumulative effects of different types of chemicals that have the potential to act on the same mechanism.

Furthermore, the researchers noted that both the active technical ingredients (i.e., glyphosate, chlorpyrifos, and lambda-cyhalothrin) and the "inactive" (claimed inert) ingredients were shown to be important, because the effects observed from the "neat" chemicals alone failed to induce uPA and were less potent inducers of uPAR\(^{39}\). Nonetheless, these pesticide formulations have been shown to be capable of increasing uPA and uPAR, which contributes to the cellular transformation that leads to tissue invasion and metastasis, an important mechanism in cancer.

The diagram below illustrates that chlorothalonil has the potential to contribute to Tissue Invasion and Metastasis via HER-2 receptor activation, and that other commercial products that are approved for use in Canada containing glyphosate, chlorpyrifos, and lambda-cyhalothrin have the potential to act via the same mode of action as well. Yet PMRA has not considered the cumulative effects of these PMRA registered chemicals in their risk assessment of chlorothalonil.
Roundup, containing glyphosate (via uPA/UPAR)

Lorsban, containing chlorpyrifos (via uPA/UPAR)

Warrior, containing lambda-cyhalothrin (via uPA/UPAR)

Chlorothalonil (via HER-2)

Again, unless PMRA assesses the cumulative effects that these combined exposures will have on this important mechanism of carcinogenicity, the margins of safety that are currently being proposed will be flawed, and the Minister will not be able to be reasonably certain that no harm will come to Canadians who are exposed to chlorothalonil (given the proposed conditions of registration).

### 2.2.4.1 Recommendations regarding Tissue Invasion & Metastasis

It is therefore recommended that PMRA ensure they have data pertaining to the potential for each of its registered products to contribute to cellular-level resistance to programmed cell death, and then factor the cumulative effects of the anticipated exposures from all of these products into its risk assessment of chlorothalonil (before proceeding with a decision to continue with the registration of the chemical) as required by the Pest Control Products Act subsection 19. (2) (b).

### 2.3 Impact of Cumulative Risk Assessment

The examples that have been provided above were intended to illustrate that there is good reason to assess the risk to human health caused by cumulative effects that are represented by all approved pesticides that share the same major carcinogenic mechanisms. While the actions of only a few of PMRA’s registered pesticides have been included in this analysis for illustrative purposes, it should be clear that the cumulative effects of the entire inventory of PMRA approved pesticides must be considered for each of these modes of action.
The diagram below illustrates how the combination of all of these individual actions has the potential to instigate cancer, and potentially erode PMRA’s estimated margins of safety.

Roundup, containing glyphosate (via uPA/UPAR)
Lorsban, containing chlorpyrifos (via uPA/UPAR)
Warrior, containing lambda-cyhalothrin (via uPA/UPAR)
Chlorothanil (via DNA Damage)
Methomyl (via DNA damage)
Ferric Sodium EDTA (DNA repair disruption)
FeHEDTA (DNA repair disruption)
Flonicamid (pathway unknown)
Malathion (via mutated p53)

Chlorothalonil (via HER-2)
Paraquat Dichloride (ROS)

2.3.1 Target Sites and Random Distribution

In the determination of whether or not the cumulative effects of multiple chemicals acting via the same mode of (carcinogenic) action should be aggregated, an argument can be marshalled to suggest that only common biological target sites should be considered. For example, chlorothalonil has been shown to generate a mutagenic adduct, in rat liver (in vivo), and measurable DNA damage in mononuclear leukocytes in farmers. So PMRA may see fit to only consider the cumulative effects of other chemicals that are known to cause genetic instability in the liver and/or in mononuclear leukocytes.

But it is important to point out that these examples of chlorothalonil’s ability to cause genetic instability in certain cell types have not been drawn from exhaustive tests that survey all possible cell types and all possible damage sites. When a chemical enters the body (by any route of exposure), it has the potential to circulate in the blood, and it is therefore foreseeable that it could have the opportunity to (disruptively) interact with almost any other tissue or substance within the body.

While it well-established that certain chemicals tend to accumulate or interact predominantly with certain organs or tissue types, this doesn’t preclude the interaction of those same chemicals in other types of tissues or in other locations within the body. And from a cumulative impact perspective, while
these other types of sites or locations may not be the norm for bodily distribution, they still represent sites where small amounts of the chemicals have the potential to contribute to the overall dose load if/when they happen to combine with other chemicals that act by the same mode of action in any given tissue/location.

3. Contaminants that will Cause Harm

The PMRA proposed decision document also notes that hexachlorobenzene, decachlorobiphenyl, pentachlorobenzene, tetrachlorobenzene, chlorinated dioxins and furans expected to be present as contaminants in the chlorothalonil technical grade active ingredients, all of which are classified as Toxic Substances Management Policy Track 1 contaminants.

The agency isolates hexachlorobenzene and explains that the current technical registrants of chlorothalonil agricultural products report that hexachlorobenzene concentrations in their current technical products are 6.0 – 8.7 ppm, which the agency claims is a 4-fold reduction from the 40 ppm previously reported (2002). PMRA also explains that HCB levels in the chlorothalonil for use only in paint preservative products are currently reported at 30 ppm which is lower than the previously reported levels of 218 to 240 ppm (1998 and 2000). The agency then concludes that this reduction in HCB in the technical active ingredients has resulted in a satisfactory decrease in the release of this contaminant into the environment which it maintains is consistent with the Toxic Substances Management Policy ultimate goal of “virtual” elimination.

However, some background is required to place this “progress” towards virtual elimination in context, because PMRA has actually failed to achieve the departments stated objectives of virtual elimination.

The Canadian Environmental Protection Act 1988 (CEPA 1988) was followed by a Priority Substances List Assessment Report on hexachlorobenzene in 1993 (jointly issued by Environment Canada and Health Canada)\(^2\). In that document it was noted that hexachlorobenzene was a persistent, and bio-accumulative, and that it had been distributed to all regions of Canada. It was estimated that >98% of the estimated intake of the substance by members of the Canadian general population was via food (primarily through such dairy products as milk, butter and ice cream), and to a lesser extent through fresh meat and eggs and peanuts/peanut butter. Furthermore, the report noted that hexachlorobenzene was known to accumulate in women’s breasts, and that the estimated intake of the substance was greater for breast-fed infants than for other age groups of the general population. The most sensitive end-point for assessment (of whether or not hexachlorobenzene was “toxic”) under paragraph 11(c) of CEPA was also noted to be carcinogenicity.

As a result, the government reviewed a number of studies that showed that hexachlorobenzene had caused several tumour types (both benign and malignant) in several species of rodents, and then used a weight of evidence approach to classify hexachlorobenzene as a Group II substance (probably
carcinogenic to man). The government further noted that hexachlorobenzene, as a Group II substance, was considered a non-threshold toxicant, meaning that it was substance for which there was some probability of harm for the critical effect (i.e., carcinogenicity) at any level of exposure, concluding that it was, therefore, considered to be "toxic" as interpreted under paragraph 11(c) of the Canadian Environmental Protection Act 1988.

The Toxic Substances Management Policy was subsequently published in 1995⁴³, and hexachlorobenzene was amongst the Track 1 contaminants identified (i.e., persistent, bio-accumulative, toxic and primarily the result of human activity). That policy targeted these substances for virtual elimination from the environment, with “virtual” elimination meaning that the goal of the policy was to ensure that Track 1 substances would not be released into the environment in measurable concentrations. Measurable release limits were to be set at the lowest concentration of a substance that can be accurately detected and quantified using sensitive but routine analytical methods⁴³. So the Minister of Health and the Minister of the Environment jointly promulgated the Prohibition of Certain Toxic Substances Regulations, 2003 (which was updated in 2005) that effectively prohibits the use, sale and import of a number of prohibited toxic substances, including hexachlorobenzene. Exceptions were granted to products containing hexachlorobenzene as a by-product at concentrations below 20ppb.

However, while all uses of hexachlorobenzene were originally scheduled for elimination as part of this broad prohibition, PMRA is noted to have expressed its concerns over regulatory redundancy, so an exemption was added to exclude them from the CEPA regulations⁴⁴. But almost 40 years have now passed since hexachlorobenzene was first removed from fungicides being used on food crops (over health concerns)⁴⁴, sixteen years have passed since the original policy to virtually eliminate hexachlorobenzene was promulgated, and eight years have now passed since the CEPA prohibition on hexachlorobenzene was in full force for all other products. Yet, PMRA is contending that the contaminant levels of hexachlorobenzene in chlorothalonil (i.e., 6–30 ppm) have been reduced and improved in a satisfactory manner. Even though these levels of contamination are actually 300-1500 times greater than the 20ppb threshold that has long been in place since 2003 for all other products in Canada under the CEPA prohibition.

Suffice it to say that PMRA has failed to implement the departments stated policy of virtual elimination of this toxic chemical. Furthermore, neither the policy, nor the execution of the policy can contradict the law, and the Pest Control Products Act states that health risks are acceptable only 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, taking into account its conditions or proposed conditions of registration [PCPA subsection 2.2)]. The act further states that the Minister’s primary objective is to prevent unacceptable risks [PCPA subsection 4.1)]. Yet, in this instance the agency is proposing to continue the registration of a number of Track 1 chemicals (in measurable quantities) that are known to be toxic/carcinogenic. So if the decision is taken to continue the registration of chlorothalonil, it will
result in these chemicals being released into the environment, which will place the Canadian population directly at risk of harm, and that is an unacceptable risk according to the Act.

Moreover, Canada is also a signatory to the Stockholm Convention, an international treaty on Persistent Organic Pollutants. Under the treaty, Canada has joined a long list of other countries and agreed to reduce or eliminate the production, use, and/or release of twelve key pollutants that are widely known as the “Dirty Dozen”. Incredibly, hexachlorobenzene, dioxins and furans are three of the twelve harmful pollutants, and they are all found in chlorothalonil. Yet, they are still being used in a manner that results in these chemicals entering the food supply. So the continued registration of chlorothalonil puts Canada’s compliance under the Stockholm Convention in question.

At the same time, the continued use of chlorothalonil presents a serious risk of harm for Canadian women and, through them, upon future generations, as well as the Arctic ecosystems and indigenous communities, which are particularly at risk because of the bio-magnification of persistent organic pollutants that occurs in this region of the globe and the resulting contamination that occurs in their traditional foods.

### 3.1 Hexachlorobenzene – Modes of Action

Hexachlorobenzene has been shown to instigate cancer by a number of modes of action. The substance disrupts the communication between cells\(^\text{45}\) which instigates sustained growth signalling, and ultimately induces cellular proliferation. It also acts on the HER-1 receptor and pathway\(^\text{46-48}\) (which has been shown to also instigate sustained growth signalling, confer resistance to programmed cell death, assist in tissue invasion and metastasis\(^\text{46,47}\), and instigate angiogenesis\(^\text{49}\)).
The diagram above illustrates that the carcinogenic contaminant, hexachlorobenzene acts on these four important modes of action (three of which are common to those instigated by chlorothalonil). This is relevant because it adds an additional layer of mechanistic complexity to chlorothalonil’s carcinogenicity when the contaminant is included, and when considered alongside the other PMRA approved pesticides that share these same modes of carcinogenic action (shown below).

Roundup, containing glyphosate (via uPA/UPAR)
Lorsban, containing chlorpyrifos (via uPA/UPAR)
Warrior, containing lambda-cyhalothrin (via uPA/UPAR)
Chlorothanil (via DNA Damage)
Methomyl (via DNA damage)
Ferric Sodium EDTA (DNA repair disruption)
FeHEDTA (DNA repair disruption)
Flonicamid (pathway unknown)
Malathion (via mutated p53)
Chlorothalonil (via HER-2)
Paraquat Dichloride (via ROS)
Hexachlorobenzene (via GJIC Inhibition)
Hexachlorobenzene (via HER-1)

In sum, hexachlorobenzene has been defined a toxic substance by the Canadian Government, one that exerts its toxicity via carcinogenic modes of action. It has been stated that the chemical is persistent and bio-accumulative and that its presence in the environment, and ongoing release into the environment, endangers Canadians. It has also been noted that there is some probability of harm for carcinogenicity at any level of exposure. Yet, in this re-evaluation of chlorothalonil, PMRA is proposing to continue to approve the use of a product that still contains dangerously high levels of this harmful contaminant which would be in violation of the Act [PCPA 2.(2)].

Furthermore PMRA has indicated that decachlorobiphenyl, pentachlorobenzene, tetrachlorobenzene, chlorinated dioxins and furans can also be expected to be found in the product, and these Track 1 chemicals are also supposed to have been eliminated as well. PMRA has not commented on the levels of these contaminants, but it is argued here that the proposed decision to approve the ongoing use of chlorothalonil, which contains hexachlorobenzene and a number of other persistent, bio-accumulative and toxic chemicals is in violation of The Pest Control Products Act which states that the Minister’s primary objective is to prevent unacceptable risks [PCPA 4.(1)].
The Act states that health risks are acceptable only 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, taking into account its conditions or proposed conditions of registration [PCPA 2.(2)]. Yet, in this instance, the government's own report has indicated that hexachlorobenzene exposures will result in some probability of harm (i.e., carcinogenicity) at any level of exposure.

3.1.1 Recommendation regarding Track 1 Contaminants

It is therefore recommended that PMRA immediately establish much stricter contamination limits for the Track 1 substances that are expected to be found in chlorothalonil before the agency makes a decision to continue with the registration of this pesticide. These limits should reflect the department's stated goal of virtual elimination as per The Toxic Substances Management Policy (1995) (i.e., measurable release limits are to be set at the lowest concentration of a substance that can be accurately detected and quantified using sensitive but routine analytical methods).

4. Failure to Protect Sensitive Groups

PMRA also has an obligation under the act [PCPA 19. (2) (b)] (ii)] to apply appropriate margins of safety to take into account, the different sensitivities to pest control products of major identifiable subgroups. But it is argued here that the proposed continuation of the registration of chlorothalonil fails to take the safety four highly-sensitive, identifiable subgroups of Canadians i.e., patients living with cancer, the overweight, pregnant women and those who are exposed in utero (as foetuses) or nursing as infants.

4.1 Canadians Living with Cancer

Unfortunately, according to the International Agency on Cancer research (IARC) Canada ranks as one of the 20 worst countries in the world from a cancer risk perspective. IARCs GLOBOCAN project aims to provide contemporary estimates of the incidence of, mortality and prevalence from major type of cancers, at national level for 184 countries of the world (http://globocan.iarc.fr). According to GLOBOCAN estimates for 2008, the risks of getting cancer in Canada are 2-4 times greater than nearly 100 other countries. According to the Canadian Cancer Society, an estimated 177,800 new cases of cancer are expected in 2011, and Stats Canada estimates suggest that there were 695,049 Canadians alive in 2005 who had been diagnosed with one or more primary invasive cancers in the previous ten years, a number that is likely to have grown slightly (given the aging demographic in Canada). So this is an enormous identifiable subgroup of Canadians who are at high risk of cancer progression or relapse/recurrence.
One of the main challenges for all cancer patients is that many of the latest treatments are targeted therapies that act on individual pathways (e.g., HER-2 receptor blocking), yet for many cancers, there are subpopulations of cancerous cells that are able to continue to proliferate using other pathways. For patients undergoing therapy, and recovering cancer patients who are living in fear of a cancer relapse, exposure to chemicals that instigate any of the major modes of carcinogenic action could be lethal.

A small percentage of cancer patients do have genetic abnormalities that predispose them to cancer (i.e., some of the hallmarks of cancer have already been instigated). Others have compromised immune systems, or other predisposing factors that place them at higher risk of cancer. Whatever the case, all of these people have cells in their bodies that are already at risk of becoming cancerous.

Yet, none of the analysis that PMRA has engaged in to arrive at safe levels of exposure for this chemical, take into account the extremely sensitive predisposition to cancer that is found within this group. For example, the addition of chlorothalonil (which acts on the HER-2 receptor) and the addition of hexachlorobenzene (which acts on the HER-1 receptor) into the diet of cancer patients receiving HER-1 and/or HER-2 receptor blocking therapy has the potential to exacerbate the progression of the cancer in these patients. It is therefore argued that PMRA has not taken the sensitivities of these Canadians into account in their development of appropriate margins of safety for chlorothalonil.

**4.1.1 Recommendation regarding Canadians Living with Cancer**

It is therefore recommended that PMRA address the issue of Canadians already living with cancer, as required by the Pest Control Products Act subsection 19. (2) (b) (i) (ii), by re-evaluating the margins of safety established for Chlorthalonil and the range of acceptable uses; and by ensuring that their calculations factor in the sensitivities of this major identifiable subgroup of Canadians.

**4.2 Overweight**

In Canada, another major identifiable subgroup that is extremely sensitive to carcinogenic chemical exposures is those who are overweight (i.e., medically defined as obese). It is estimated that more than 25% of the Canadian population (roughly 8-9 million) fall into this medical category\(^5^0\), so this is a substantial cohort of Canadians who have an elevated level of cancer risk.

The mechanistic relevance of obesity to cancer is slowly being elucidated by researchers. For example, some researchers have made convincing arguments that tie obesity to a pro-inflammatory state where chronic inflammation instigates many of the major mechanisms that can contribute to the disease\(^5^1\) (i.e., genetic instability, sustained proliferative signalling, evasion of anti-growth signalling, resistance to programmed cell death, replicative immortality, promotion of angiogenesis, invasion and metastasis\(^5^2\)).
Other research has shown that additional contributing factors may also link obesity to cancer\textsuperscript{53,54} (such as high levels of insulin-like growth factor (IGF-I), which can promote sustained proliferative signalling\textsuperscript{55}, resistance to programmed cell death, tissue invasion and metastasis\textsuperscript{56}).

In sum, these are additional linkages that represent endogenous chemical influences that place this group at higher risk of getting cancer (depicted below).

![Diagram of obesity and cancer linkages](image)

When these predispositions are combined with chlorothalonil, hexachlorobenzene, and other PMRA approved pesticides that impact the same major mechanisms as chlorothalonil, the potential for cumulative contributions are significant (illustrated below).
Roundup, containing glyphosate (via uPA/UPAR)
Lorsban, containing chlorpyrifos (via uPA/UPAR)
Warrior, containing lambda-cyhalothrin (via uPA/UPAR)
Chlorothanil (via DNA Damage)
Methomyl (via DNA damage)
Ferric Sodium EDTA (DNA repair disruption)
FeHEDTA (DNA repair disruption)
Flonicamid (pathway unknown)
Malathion (via mutated p53)
Chlorothalonil (via HER-2)
Paraquat Dichloride (via ROS)
Hexachlorobenzene (via GJIC Inhibition)
Hexachlorobenzene (via IGF-1)
Obesity (via Inflammation)
Obesity (via IGF-1)

Yet, in this proposed registration for chlorothalonil, no measures have been taken to ensure that the carcinogenicity exposure testing in rodents is relevant to this high-risk segment of the population. Nor have any of the PMRA risk estimates taken the predispositions of this high risk group into account.

Yet the Minister has a responsibility to ensure that the carcinogenic health risks associated with pesticides that are being approved are understood and that appropriate margins of safety are used to take the sensitivities of this group into account. Otherwise, the agency cannot be reasonably certain that no harm will come to the 8-10 million Canadians who fall into this category.

4.2.1 Recommendation regarding those who are Overweight

It is therefore recommended that PMRA revisit the test requirements, the risk assessment methods, and the margins of safety that have been established for this product, and ensure that the agency is using an approach that factors the sensitivities of the 8-9 million Canadians that fall into this category into account (as required by the Act [19. (2) (b)] (iii) ).
4.3 Pregnant Women and Nursing Mothers

Another major identifiable subgroup that is extremely sensitive to carcinogenic chemical exposures is that of pregnant women, and nursing mothers. A 2010 study conducted by the Centers for Disease Control and Prevention in the United States tested maternal and umbilical cord serum in a cohort of 150 women in the United States and chlorothalonil was noted as one of a number of pesticides that was detected\(^57\), and hexachlorobenzene is also known to accumulate in the breast\(^42\). So it can be assumed that pregnant women and nursing mothers will be exposed to both of these substances if the proposed decision to continue the registration of chlorothalonil is approved.

However, as noted above, PMRA has not considered the impact of the cumulative effects of chlorothalonil combined with other registered pesticides that act via the same carcinogenic mechanisms, and the agency knows that chlorothalonil contains measurable amounts of hexachlorobenzene which has been deemed a carcinogenic risk at any level of exposure.

From a carcinogenicity perspective, the robust literature of the intergenerational effects and damage of diethylstilbestrol on daughter generations should be also considered as well. The intergenerational mechanisms of DES induction of cancer and/or the low dose effects of disruption of normal female cycling by a PCB congener could very well apply to chlorothalonil or the contaminants in its formulation. However, no evaluation on the potential that Chlorthalonil might cause cancer in the offspring has been conducted. Furthermore, chlorothalonil is also expected to contain chlorinated dioxins, and PMRA has not evaluated or employed testing for low or moderate dose exposures of this sort (even though many chemicals of similar structure cause intergenerational malfunction of reproductive processes through endocrine signalling that disrupts critical cellular functions\(^58\)), so the intergenerational effects of chlorothalonil and it’s contaminants are untested and the effect of the intergenerational effects of these chemical on Canadians remain unknown.

Therefore, if the proposed decision to continue to register chlorothalonil goes forward, pregnant women, those who are susceptible to being exposed in utero (as foetuses), and nursing infants will all be facing an unacceptable level of risk (as defined by the subsection 2 (2) of the Pest Control Products Act).

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<tr>
<th>4.3.1 Recommendation regarding Pregnant Women and Nursing Mothers</th>
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<td>It is therefore recommended that PMRA insist upon a greater range of testing for low or moderate dose exposures that are known for many chemicals of similar structure to cause intergenerational malfunction of reproductive and developmental processes through endocrine signalling that disrupts critical cellular functions. In particular, the ability of chlorothalonil to impact pregnant women, nursing</td>
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mothers, and potentially predispose children (in utero or via mother’s milk) to cancer needs to be carefully investigated. The agency should revisit the established practices for testing, the assumptions that have been made and the margins of safety that have been established for this product, and ensure that the methods being employed factor the sensitivities of this major identifiable subgroup of Canadians into account as required by the Pest Control Products Act subsection 19. (2) (b) (ii)

4.4 Those who Smoke

Finally, and somewhat unfortunately, in Canada another major identifiable subgroup that is extremely sensitive to carcinogenic chemical exposures is the large cohort of Canadians who smoke tobacco products. According to Stats Canada, in 2010, 20.8 per cent of Canadians aged 12 and over were still being counted as smokers (or roughly 6 million people), so this major subgroup of the population that is predisposed to cancer in a very obvious way.

Therefore PMRA has a responsibility to ensure that the additional carcinogenic risks associated with the ongoing use of chlorothalonil, or any other pesticide that is being approved for use, take the cancer-related sensitivities of this group into account. Although, some might argue that smokers have chosen to place themselves at risk, the government of Canada considers the sale of tobacco products as lawful (despite the addictiveness of these products and the well-established role that they have in cancer causation), and it profits directly from the taxes earned on the sale of these products, so Health Canada has an obligation to ensure that those who smoke are not discriminated against.

Yet none of the testing or evaluation procedures that that PMRA has employed in their assessment of chlorothalonil factors the sensitivities of the 6 million Canadians in this category into account. Therefore, the Minister cannot be reasonably certain that no harm will come to those who fall into this category if they are also exposed to chlorothalonil under the proposed conditions of use.

4.4.1 Recommendation regarding those who Smoke

It is recommended that PMRA revisit the margins of safety established for this product, and the range of acceptable uses and ensure that their calculations factor the sensitivities of the roughly 6 million Canadians who smoke into account as required by the Pest Control Products Act subsection 19. (2) (b) (ii)
4.5 Other Relevant Factors

The Pest Control Products Act [19 (2)] gives the Minister considerable latitude to establish acceptable methods for the evaluation of health risks posed by a pest control product during a re-evaluation in the determination as to whether or not those risks are acceptable. However, the Minister is tasked to ensure that a scientifically based approach is taken, and to consider relevant factors [19 (2)(b)(i)]. As noted above, the science of cancer has evolved considerably since PMRA’s evaluation methods for carcinogenicity determination were first developed. As a result, the agency appears to have missed a number of relevant factors that should have been factored into any assessment relating to the carcinogenic potential of a chemical.

The World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) Workshop on Aggregate/Cumulative Risk Assessment (Combined Exposures to Multiple Chemicals) was held in Washington, DC, USA, on 19–21 March 2007. This workshop is important because one of the the principal objectives of the activity, which involved experts from agencies worldwide, was to consider the state of the art of the science related to cumulative risk assessment.\(^2\)

The WHO/IPCS approach to the assessment of the co-occurrence of, and concomitant exposure to, multiple chemicals explains that the decision to consider compounds in an assessment group is commonly based on information indicating that the components are believed to act similarly or interact, and notes that this decision can be based on biological data that lead to the conclusion that effects are likely to be similar.\(^2\) In this instance, as we evaluate the potential for various chemicals to contribute to cancer causation, PMRA knows with certainty that many of the chemicals that it approves enter the food supply and will be consumed by Canadians on a daily basis. Furthermore, we are fortunate to have an enormous and robust literature of data that supports the rationale for each of the hallmarks of cancer as important modes of action that are all inter-related, so we know with certainty that each of these modes of action can contribute to disease causation.

Therefore, at a minimum, the agency should be considering the cumulative effects of all of its approved pesticides with respect to their ability to collectively act upon each of the hallmarks of cancer (i.e., not just assess the cumulative effects of the individual modes of action that are shared in common with any single pesticide). While this approach is not explicitly stated in the act, any scientifically-based policy that does not consider the aggregated influence of chemicals that can individually act on any one of these modes of action would be conceptually flawed.

With this in mind, the following sections of this document address the importance of the remaining hallmarks of cancer (i.e., those that have not been discussed thus far):
4.5.1 Inflammation

The Hallmarks of Cancer framework highlights two important enabling modes of action which are Genetic Instability and Inflammation (shown below). Factors that contribute to genetic instability have long been known to contribute to cancer, however, chronic inflammation is also notorious in its ability to instigate many of the major mechanisms that can contribute to the disease (i.e., genetic instability, sustained proliferative signalling, evasion of anti-growth signalling, resistance to programmed cell death, replicative immortality, promotion of angiogenesis, invasion and metastasis), yet PMRA has undertaken no assessment of chlorothalonil’s ability to instigate these other hallmark mechanisms via this very important pathway.

4.5.1.1 Hypothalamic-Pituitary-Adrenal Axis Disruption

One important means by which chemicals can instigate chronic inflammation is via disruptions to the hypothalamic-pituitary-adrenal axis (i.e., stress-axis). When cortisone is needed to suppress inflammation anywhere in the body, the hypothalamus releases a chemical called corticotrophin-releasing factor (CRF). In turn, the presence of CRF stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) into the blood. ACTH then induces the secretion of cortisol from the adrenal cortex in the adrenal glands, and the cortisol that is produced by the stress-axis is then circulated throughout the body and converted to cortisone when needed.
The stress-axis is a diurnal system that offers peak production of cortisol in the morning and levels of cortisol fade over a 24 hour period. As cortisol levels first become elevated above normal baseline levels, circulating levels of the master inflammatory cytokine Macrophage migration Inhibitory Factor (MIF) become elevated as well\(^5\). Yet when levels of MIF become excessive, even higher levels of cortisol are then produced to suppress the resulting inflammation\(^5\). In other words, the stress-axis can act both to instigate the inflammatory response (when slightly elevated levels of cortisol are produced) and to suppress the inflammatory response (when significantly elevated levels of cortisol are produced). Therefore disruptive chemical exposures that interfere with this hormonal cascade, or damage the pituitary gland, or adrenal gland, and/or chemicals that mimic the effects of MIF by acting on the MIF receptor (CD74)\(^6\), can negatively impact a person’s ability to suppress inflammation, and have a direct role in instigating tumor-promoting inflammation\(^62-64\).

It is notable then that a risk characterization review for dietary exposures to chlorothalonil was undertaken by the California Environmental Protection Agency in 2005 discussed the fact that Beagle dogs that were fed chlorothalonil for 13 weeks showed treatment-related hypertrophy of the zona fasciculata of the adrenals (seen in both sexes in the high dose groups)\(^65\). This is relevant because the zona fasciculata in the adrenal gland is responsible for cortisol production, so it is the sort of disruption that could impair an individual’s ability to suppress inflammation.

While the dose levels in this particular study were many times over those that would be expected at the population level if chlorothalonil continues to be registered, PMRAs risk assessment is lacking because the agency has not factored in the cumulative effects that all of its registered pesticides may be having on this very important mode of action.

For example Diazinon\(^66\) is another PMRA registered insecticide used on a wide variety of food and feed crops, and has been shown to be capable of killing adrenal cells and disrupting the production of adrenal steroid hormones\(^67\) as well. Yet PMRA does not know whether or not the ongoing use of chlorothalonil, combined with Diazinon (and any other PMRA registered chemical that has the potential to disrupt the stress axis) will impair cortisol production at the population level, because the agency has not factored the cumulative effects of these chemicals and their impact on inflammation into its risk assessment.

Furthermore PMRA has done no testing to determine what the low dose effects of chlorothalonil might be on the stress-axis for anyone exposed in utero or neonatally via breast milk. For example, mice exposed to low doses of Diazinon in utero and neonatally via mothers’ milk were found to have persistently elevated plasma levels of corticosterone, yet the high dose groups in the same study did not exhibit the same sort of dysregulation\(^68\). This example is offered because this sort of non-linear dose-response defies the assumptions that form the basis upon which PMRAs safe exposure level estimates were made. Yet the agency has no low dose data to determine the sort of disruptive effects that chlorothalonil might have on the stress-axis.

Therefore PMRA does not know whether or not the ongoing use of chlorothalonil has the potential to disrupt the stress axis, or MIF levels of children exposed in utero, nor of nursing infants who are exposed
to chlorothalonil in breast milk. So the agency cannot be reasonably certain that chlorothalonil will be safe to use at the proposed levels.

4.5.1.2 Chronic Infection

A second common means by which inflammation can be induced is by persistent chronic infection. This can be the result of an inadequate immune system response which will be discussed in greater detail below.

4.5.1.3 Recommendations on Inflammation

Given the Minister’s responsibility to ensure that a scientifically based approach is taken [PCPA 19 (2)(b)(i)], and given the fact that that chronic inflammation is now widely recognized for its mechanistic contribution to cancer (and therefore a highly relevant factor), it is suggested that PMRA insist upon test data for chlorothalonil that can establish with reasonable certainty that it is not disrupting the endocrine hormones that are secreted as messengers within the stress-axis, and that it is not disrupting the function of the endocrine producing glands within the stress axis. Also, PMRA should take steps to ensure that it understands the intergenerational effects on this capability that the chemical imposes at low dose exposures, since this sort of disruption can occur in a non-linear fashion.

Furthermore, if it is ascertained that any of these disruptive effects are occurring, PMRA should also insist upon similar data for all of its other registered products, and a cumulative assessment of the combined impact of all chemicals having a similarly disruptive ability on this system should be undertaken prior to making the decision to continue with the registration of chlorothalonil.

4.5.2 Evasion of Anti-growth Signalling

Another key cellular function that is supposed help prevent unwarranted cellular proliferation is anti-growth signalling. At any given point in time, it may be important for the cell not to grow, divide and replicate, so all cells have machinery that is supposed to prevent this from happening at inopportune times.

For example, to maintain genome stability, cells with damaged DNA must ensure that cellular growth is arrested while DNA repair is taking place. The retinoblastoma tumour suppressor protein (referred to as pRb) is best known for its role in this important function, but this key protein is frequently inactivated in human cancers. This sort of inactivation of pRb can occur when DNA is damaged. However it can also occur when it is disrupted by certain chemicals. For example, a number of notoriously carcinogenic pesticides (e.g., heptachlor, chlor dane, and toxaphene) specifically disrupt pRb function. This is not
the only manner by which this mode of action can be enabled but this example is offered here for illustrative purposes.

However, PMRA has no regulatory criteria that specifically relate to the cumulative effects that the individual contributions of its registered pesticides might have on cells that would allow them to evade anti-growth signalling. This is an important hallmark mode of action that is known to contribute to cancer causation so an evaluation of this sort would be warranted in any scientifically based program that hoped to truly understand the cancer risks associated with a multitude of combined low dose chemical exposures.

### 4.5.2.1 Recommendations regarding Evasion of Anti-growth Signalling

Given the Minister’s responsibility in the Pest Control Products Act subsection 19 (2)(b)(i) to ensure that a scientifically based approach is taken, and given the fact that that the Evasion of Anti-growth Signalling is now widely recognized for its mechanistic contribution to cancer, it is recommended that PMRA consider the effects that chlorothalonil has on the ability of cells to evade anti-growth signalling, and also assess cumulative effects of all of its other registered pesticides on this same capability. This analysis should take into account any relevant intergenerational effects via in utero exposure or on nursing infants. And these precautionary measures should be undertaken before any decision is made regarding the continued registration of chlorothalonil.

### 4.5.3 Replicative Immortality

Another important safeguard that should stop proliferating cells from becoming immortalized is a practical limit that has been established within all cells to prevent endless replication. A structure on the end of the chromosomes called a telomere is reduced in length each time a cell makes a copy of itself, and when the telomere is shortened to a critical length, a process called replicative senescence forces the cell into a state of permanent growth arrest. So senescence is yet another important tumour suppressor that should prevent the proliferation of seriously damaged cells.

However, the immortalized cells that are proliferating in cancer have found a way to bypass this safeguard. This capability can be rendered dysfunctional when DNA is damaged, but it can also be manipulated chemically. For example, elevated levels of an enzyme called telomerase can extend the length of telomeres on the chromosomes, which can delay senescence, and allow cellular proliferation to continue when it would otherwise be stopped, and elevated levels of the hormone leptin are known to be capable of elevating the levels of this important enzyme.

However, PMRA has no regulatory criteria that specifically relate to the cumulative effects that the individual contributions of its registered pesticides might have on cells that would allow them to evade achieve replicative mortality. This is a hallmark mode of action that is known to contribute to cancer
causation so an evaluation of this sort would be warranted in any scientifically based program that hoped to truly understand the cancer risks associated with a multitude of combined low dose chemical exposures.

### 4.5.3.1 Recommendations regarding Replicative Immortality

Given the Minister’s responsibility to ensure that a scientifically based approach is taken [PCPA 19 (2)(b)(i)], and given the fact that that the Replicative Immortality is now widely recognized for its mechanistic contribution to cancer, it is recommended that PMRA consider the effects that chlorothalonil has on the ability of cells to achieve replicative mortality, and also assess cumulative effects of all of its other registered pesticides on this same capability, taking into account the intergenerational effects, in utero exposures, and the impact on nursing infants. And these precautionary measures should all be undertaken before any decision is made regarding the continued registration of chlorothalonil.

### 4.5.4 Avoiding Immune Destruction

Another very relevant factor in any assessment of carcinogenicity is immune system function. The immune system is a multi-pronged and highly potent line of defence that can eradicate tumors, and it consists of a great number of important cell types that can suppress proliferating cells that are forming tumors. T-cells, Natural Killer cells, macrophages and dendritic cells all have an important role to play in tumor eradication, and also in the clearance of infections (that can cause chronic inflammation if not cleared). Proliferating cells that are able to avoid immune system destruction are therefore a serious problem.

Yet a great number of PMRA registered pesticides have been shown to have disruptive (i.e., immunosuppressive potential). For example, low doses of chlorpyrifos have generally been shown to disrupt the immune system. Researchers have shown that chlorpyrifos can selectively kill and/or disrupt lymphocytes, such as T-cells and natural killer cells. While other researchers have shown that when newborn mice are exposed to low doses of chlorpyrifos that their T-cell responses appear normal when they are young but they become impaired as the mice get older.

Since PMRA does not consider the cumulative effects of chlorothalonil, chlorpyrifos, and all of its other registered pesticides on the immune system of adults, foetuses in the womb or neonates, the agency does not have the ability to determine the extent to which the immune system of the population may have been compromised by the cumulative effects of these low dose exposures.

Yet this is an extremely relevant mode of action that must be assessed. If the cumulative effects of low dose exposures to all PMRA registered pesticides are impairing an aspect of the immune system function of the Canadian population, then their level of sensitivity to other disruptive chemicals with carcinogenic potential will increase considerably. Therefore the agency cannot be reasonably certain.
that the population will not be harmed by the ongoing exposures to chlorothalonil that are being proposed.

### 4.5.4.1 Recommendations on Avoiding Immune System Destruction

Given the Minister’s responsibility to ensure that a scientifically based approach is taken [PCPA 19 (2)(b)(i)], and given the fact that the immune system is now widely recognized for its contribution to cancer, it is recommended that PMRA consider the effects that chlorothalonil has on the various relevant parts of the immune system (e.g., The Thymus, T-cells, Natural Killer cells, macrophages and dendritic cells) and also assess cumulative effects of chlorothalonil, chlorpyrifos, and all of its other registered pesticides on the immune system, also taking into account any relevant intergenerational effects, in utero exposures, and nursing infants. This should be undertaken before any decision is made regarding the continued registration of chlorothalonil.

### 4.5.5 Angiogenesis

Angiogenesis, is another important mode of action in cancer whereby new blood vessels grow from the existing vasculature, to supply more oxygen and nutrition to a tumor. Angiogenesis can be switched on by growth factors secreted by tumor cells such as Vascular Endothelial Growth Factor (VEGF). In response to this signaling, new blood vessels sprout from the existing vasculature and grow in and around the tumor to supply the needed blood to the oxygen-deprived cells. The manipulation of VEGF is so important that numerous anti-angiogenic therapeutics have been developed to treat cancer. However, PMRA has no regulatory criteria that specifically relate to the cumulative effects that the individual contributions of its registered pesticides might have on angiogenesis. This is another important mode of (carcinogenic) action so an evaluation of this sort would be warranted in any scientifically based program that hoped to truly understand the cancer risks associated with a multitude of combined low dose chemical exposures.

### 4.5.5.1 Recommendations regarding Angiogenesis

Given the Minister’s responsibility to ensure that a scientifically based approach is taken [PCPA 19 (2)(b)(i)], and given the fact that the immune system is now widely recognized for its contribution to cancer, it is recommended that PMRA consider the effects that chlorothalonil has on angiogenesis, and also assess cumulative effects of all of its other registered pesticides on this same capability. This should be undertaken before any decision is made regarding the continued registration of chlorothalonil.
5. Summary / Conclusions

In summary, a number of major concerns have been raised in this submission.

First of all, a number of modes of carcinogenic action have been documented both for chlorothalonil, and the contaminants that are known to be found in the product (e.g., hexachlorobenzene), and there are other PMRA registered pesticides that have been shown to exert their carcinogenic potential via the same modes of action. Yet PMRA has failed to consider the cumulative effects of those other pest control products as required by the Pest Control Products Act [19. (2) (b)]. This means that PMRA estimates of safe levels of exposure to chlorothalonil are likely to underestimate the true risks of exposure. The agency therefore cannot be reasonably certain that no harm to human health will occur if the registration of the product is continued as proposed, which is an unacceptable level of risk1.

In the second instance, PMRA has explained that hexachlorobenzene, and a number of other Track 1 contaminants can be expected to be present in the chlorothalonil technical grade active ingredients. Yet the Toxic Substances Management Policy scheduled these persistent, bio-accumulative and toxic substances for virtual elimination in 199543, and no measureable amounts of these substances are to be allowed into the environment. Yet sixteen years have now passed, and PMRA has failed to comply with this important policy. Furthermore, hexachlorobenzene has been classified as a Group II substance that is considered a non-threshold toxicant, meaning that there is some probability of harm for the critical effect (i.e., carcinogenicity) at any level of exposure. So this 16 year delay in implementing government policy represents an ongoing practice that is likely to cause harm to Canadians, in violation of The Pest Control Products Act subsection 4.(1) which states that the Minister’s primary objective is to prevent unacceptable risks.

In the third instance, it has been shown that PMRA’s proposed margins of safety for exposure to chlorothalonil have not taken into account the sensitivities of a number of major identifiable subgroups within the Canadian population who are at risk of cancer (i.e., Canadians living with cancer, those who are medically defined as obese, those who smoke, pregnant women, foetuses exposed in utero, and nursing infants) as required by the Pest Control Products Act subsection 19(2)(b)(ii). The Minister therefore cannot be reasonably certain that no harm to human health will come to these Canadians.

Moreover, PMRA’s risk estimates are not based on the sorts of low dose tests that can offer insights into the effects of this chemical and its contaminants on intergenerational carcinogenicity, nor of

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1 Where health risks are acceptable only if there is reasonable certainty that no harm to human health will result from exposure to or use of the product, taking into account its conditions or proposed conditions of registration as per PCPA subsection 2.(2)
malfunction of reproductive processes through endocrine signalling that disrupt critical cellular function\textsuperscript{58}. Therefore PMRA’s risk assessment methods have not been adequately developed as they do not take into account the different sensitivities to pest control products of those who fall into these categories either (as required by the Pest Control Products Act subsection 19(2)(b)(ii)).

Finally, PMRA has not considered a number of other important hallmark modes of carcinogenic action into account, even though they are widely agreed to be relevant (i.e., inflammation, evasion of antigrowth signalling, replicative immortality, avoidance of immune system destruction, angiogenesis). Nor has the agency considered the extent to which other chemicals that have already been registered might cumulatively act on each of these modes of action. But the Pest Control Products Act requires the Minister to ensure that a scientifically based approach is taken to risk assessment and to consider relevant factors [PCPA subsection 19 (2)(b)(ii)]. So the methods being employed by PMRA need considerable refinement and updating, because the agency is not able to account for the mixture effects of the many low dose chemical exposures that result from PMRAs long list of registered pesticides.

In sum, PMRAs proposed re-evaluation decision for the fungicide chlorothalonil (Proposed Re-evaluation Decision PRVD2011-14, 1 Nov 2011)\textsuperscript{1} is a decision that is premature, and likely to cause harm to Canadians. Continuing to register chlorothalonil would therefore place the agency in violation of the Pest Control Products Act in several respects. As a result, a series of recommendations have been made in this document that will allow PMRA to better assess the carcinogenic potential of this chemical, its contaminants (and other chemicals) in the future.

However, until the actions prescribed in these recommendations have been undertaken, the agency cannot be reasonably certain that no harm to human health will occur if the registration of the product is continued as proposed. In fact, given what is known about the Track 1 contaminants that are known to be included in the chlorothalonil technical product, PMRA can be certain that harm to Canadians is occurring, and will continue to occur (so long as chlorothalonil, and its contaminants continue to be approved for use). Therefore its continued registration (as is proposed) would represent an unacceptable level of risk and an unlawful action by the Minister (i.e., in contravention of the PCPA which is constitutional under the Criminal Law Power).

We therefore implore the Minister to use the precautionary principle as described in section 20 (1) of the Pest Control Products Act. The Pest Control Products Act says that, when in the course of a re-evaluation of a pesticide an amendment or cancellation is necessary to protect human health and safety,

20. (1) the Minister may cancel or amend the registration of a pest control product,

(b) in the course of a re-evaluation of special review, the Minister has reasonable grounds to believe that the cancellation or amendment is necessary to deal with a situation that
endangers human health or safety or the environment, taking into account the precautionary principle set out in subsection (2) [emphasis added]

(2) where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent adverse health impacts or environmental degradation. [emphasis added]

The precautionary principle has been accepted and applied by the Supreme Court of Canada. In reference to the principle, the Court has stated:

Canada ‘advocated inclusion of the precautionary principle’ during the Bergen Conference negotiations (D. Vander Zwaag, CEPA Issue Elaboration Paper No. 18, CEPA and the Precautionary Principle/Approach (1995), at p. 8). The principle is codified in several items of domestic legislation: see for example the Oceans Act, SC 1996, c. 31, Preamble (para. 6); Canadian Environmental Protection Act, 1999, SC 1999, c. 33, s. 2(1)(a); Endangered Species Act, SNS 1998, c. 11, ss. 2(1)(h) and 11(1).

Scholars have also documented the precautionary principle’s inclusion ‘in virtually every recently adopted treaty and policy document related to the protection and preservation of the environment’ (D. Freestone and E. Hey, ‘Origins and Development of the Precautionary Principle’, in D. Freestone and E. Hey, eds, The Precautionary Principle and International Law (1996), at p. 41).²

Moreover, DeMarco and Campbell state:

… at the time that the [Supreme Court of Canada] embraced the precautionary principle in the Hudson Case, international acceptance of the concept was well developed, but Canadian domestic implementation of it was relatively limited. Since that decision was released, nearly every new federal law affecting the environment has referred to the principle in some way.³

Notably, the importance of applying the precautionary principle is heightened when there is evidence that indicates that the current state of affairs raises immediate concerns of harm to the public (as is the case with the ongoing use of chlorothalonil and its contaminants). The minister is therefore urged to discontinue the registration of chlorothalonil immediately, as she is empowered to do by the PCPA subsection 20. (1) (b).

Alternatives are available to this product as there is currently a full roster of fungicides that are used to control rust on the same crops. Also other methods to control rust, such as intensive Integrated Pest...

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² 114957 Canada Ltée (Spraytech, Société d’Arrosage) v. Hudson (Town), [2001] 2 SCR 241
Management programs, crop rotation, crop diversification, innovative irrigation, and resistant varieties should be promoted to sustain yields while greatly reducing the need for fungicides of this nature.

In closing, it should be added that it is sadly ironic that the Department of Health is proposing to allow the continued use of chlorothalonil and hexachlorobenzene, when both chemicals are known carcinogens that have been shown to be capable of activating the HER-2 and HER-1 cellular receptors respectively. Because these chemicals are in our food and our water, and ultimately end up in our bodies\textsuperscript{57,81,82}, and Health Canada and the provinces are now sharing the costs for Trastuzumab, a HER-2 receptor blocking breast cancer therapy that has been estimated to add an estimated $127 million in new burden to Canada's health care system each year\textsuperscript{83}. Moreover, new breast cancer therapy that blocks the activation of both HER-1 and HER-2 receptors (i.e., Lapatinib\textsuperscript{84}) has already been approved for use in Nova Scotia and Saskatchewan. So the very idea that the Government of Canada would allow these HER-1 and HER-2 activating carcinogens to continue to be used in a manner that ensures that they end up in our food and water is an affront to all Canadians.

Why should Canadians have to live in an environment where the risks of getting cancer are nearly the highest in the world?

Our families deserve better.


59. Isidori AM, Kaltsas GA, Korbonits M, Pyle M, Gueorguiev M, Meinhardt A et al. Response of serum macrophage migration inhibitory factor levels to stimulation or suppression of the


Appendix A

No Significant Risk Level (NSRL) for the Proposition 65 Carcinogen Chlorothalonil

March 2011

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency
SUMMARY OF FINDINGS

The human cancer potency of chlorothalonil was estimated and used to calculate a “No Significant Risk Level” (NSRL). The human cancer potency was estimated from dose-response data for multiple treatment-related tumor sites in male Fischer 344 rats exposed via their feed (Wilson and Killeen, 1989). An overall estimate of cancer potency associated with all treatment-related renal and forestomach tumors observed in the study was derived using a multisite statistical approach. The potency derivation takes into account differences in body size between humans and experimental animals. The human cancer potency estimate for chlorothalonil is 0.026 (mg/kg-day)^{-1}.

The Proposition 65 NSRL is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The NSRL for chlorothalonil is calculated to be 27 μg/day.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Cancer Potency (mg/kg-day)^{-1}</th>
<th>NSRL (μg/day)</th>
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<tbody>
<tr>
<td>Chlorothalonil</td>
<td>0.026</td>
<td>27</td>
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INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for chlorothalonil (CAS No. 1897-45-6). Chlorothalonil was listed on January 1, 1989, as known to the State to cause cancer under Proposition 65 (formally known as the Safe Drinking Water and Toxic Enforcement Act of 1986; California Health and Safety Code 25249.5 et seq.).

Chlorothalonil is used in agriculture and horticulture as a fungicide, bactericide, and nematicide. It is also used as a preservative in wood, paints, and adhesives. Chlorothalonil is not known to occur naturally (IARC, 1999).

The studies available for cancer dose-response assessment and the derivations of the cancer potency estimate and NSRL are discussed below. A detailed description of the methodology used is provided in the Appendix.
STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

There are no human cancer epidemiology studies of chlorothalonil. Several long-term animal cancer bioassays in rats and mice employing dietary administration of chlorothalonil have been conducted:

- The National Cancer Institute (NCI) conducted 80-week cancer bioassays of chlorothalonil in Osborne-Mendel rats and B6C3F1 mice of both sexes (NCI, 1978).
- Bio/dynamics Inc., conducted two-year cancer bioassays in Charles River CD-1 mice of both sexes (Wilson et al., 1983; Wilson et al., 1986; Wilson and Killeen, 1986; all as reported in California Department of Pesticide Regulation [CDPR], 2005).
- The International Research and Development Corporation (IRDC) conducted 27-month and 30-month cancer bioassays in male and female Fischer 344 rats, respectively (Wilson, 1985 [males]; Wilson, 1986 [females]; both as reported in CDPR, 2005), and a two-year cancer bioassay in Charles River CD-1 male mice (Wilson and Killeen, 1987, as reported in California Department of Food and Agriculture [CDFA], 1988 and CDPR, 1998).
- IRDC and Experimental Pathology Laboratories, Inc. (IRDC/EPL) conducted 23-to 26-month and 29-month cancer bioassays in male and female Fischer 344 rats, respectively (Wilson and Killeen, 1989).
- Huntingdon Life Sciences conducted 80-week cancer bioassays in Crl:CD-1® (IRC)BR mice of both sexes and two-year cancer bioassays in Crl: CD®(SD)BR rats of both sexes (Spencer-Briggs, 1995a [mice]; Spencer-Briggs, 1995b [rats], as reported in CDPR, 1997a [mice] and CDPR, 1997b [rats]).

In the NCI studies in Osborne-Mendel rats, groups of 10 animals/sex/control group and 50 animals/sex/treatment group were administered 0, 253 and 506 mg/kg-day chlorothalonil via their feed for 80 weeks and observed for an additional 30 weeks (NCI, 1978). Renal adenomas and carcinomas were observed in males (0/10, 2/46, and 1/49 for control, low- and high-dose groups, respectively) and females (0/10, 0/48, and 3/50 for control, low- and high-dose groups, respectively) but there were no statistically significant increases in treatment-related tumors.

In the NCI studies in B6C3F1 mice, groups of 10 animals/sex/control group and 50 animals/sex/dose group were administered 0, 384, and 768 mg/kg-day (males) and 0, 429, and 851 mg/kg-day (females) chlorothalonil via their feed for 80 weeks and observed for an additional 11-12 weeks (NCI, 1978). No statistically significant increases in the incidence of treatment-related tumors were observed.

In the Bio/dynamics Inc. studies in Charles River CD-1 mice, 60 animals/sex/group were administered 0, 127, 265, and 551 mg/kg-day chlorothalonil via their feed for two years (Wilson et al., 1983; Wilson et al., 1986; Wilson and Killeen, 1986, as reported in CDPR, 2005). Papillomas and carcinomas of the forestomach were observed in both sexes (0/57, 2/60, 5/53, and 2/50 in males; 0/52, 2/57, 5/54, and 5/51 in females), with statistically significant increases for mid-dose males (p < 0.05) and for mid- and high-dose females (p < 0.05 in both cases) as well as a significant overall trend in females (p < 0.05). Additionally, renal adenomas and carcinomas were observed in male mice.
(combined incidence: 0/57, 6/60, 4/53, and 5/50) with significant increases in the low- and high-dose groups (p < 0.05 in both cases).

In the IRDC studies in Fischer 344 rats, 60 animals/sex/group were administered 0, 40, 80, and 175 mg/kg-day chlorothalonil via their feed for 27 months (males) and 30 months (females) (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005). Papillomas and carcinomas of the forestomach were observed in treated rats of both sexes with significant trends (males: p < 0.05; females: p < 0.001), with a statistically significant increase in high-dose females as compared to controls (p < 0.01) (see Table 2). Statistically significant increases in renal adenomas and carcinomas were also observed in male and female rats of both sexes at all dose levels except low-dose females, as compared to controls (Table 2), with significant trends for both sexes (p<0.0001).

In the IRDC study in Charles River CD-1 male mice, groups of 60 animals/group were administered 0.0, 1.86, 5.35, 23.2, and 99.7 mg/kg-day chlorothalonil via their feed for two years (Wilson and Killeen, 1987, as reported in CDFA, 1988, and CDPR, 1998). No statistically significant increases in the incidence of treatment-related tumors were observed.

In the IRDC/EPL studies in Fischer 344 rats, 55 animals/sex/group were administered 0, 2, 4, 16, and 182 mg/kg-day chlorothalonil via their feed for 99 weeks in high-dose males, 111 weeks for all other males, and 125 weeks for all females (Wilson and Killeen, 1989). Papillomas and carcinomas of the forestomach were observed in female rats with a significant trend (p<0.001) and with a statistically significant increase in high-dose females as compared to controls (p < 0.01) (see Table 3). Papillomas of the forestomach were observed in male rats with a significant trend (p<0.01) and with a statistically significant increase in high-dose males as compared to controls (p < 0.05) (Table 3). Renal adenomas and carcinomas were also observed in males and females with significant trends (p < 0.0001) and with statistically significant increases in high-dose rats of both sexes (p < 0.0001) (Table 3).

In the Huntingdon Life Sciences studies in Crl:CD-1® (IRC) BR mice, 50 animals/sex/group were administered 0.0, 2.2, 8.9, 35.5, and 143.5 mg/kg-day chlorothalonil via their feed for 80 weeks (Spencer-Briggs,1995a, as reported in CDPR, 1997a). Epithelial hyperplasia of the non-glandular forestomach and the limiting ridge was increased in male mice at all dose levels. Squamous cell papillomas of the non-glandular forestomach were observed in males (1/50, 0/50, 0/50, 2/50, and 4/50) with a significant trend (p < 0.01) and females (0/50, 0/50, 1/49, 0/50, and 5/50) with a significant trend (p < 0.0001), and with a statistically significant increase in high-dose females (p < 0.05).

In the Huntingdon Life Sciences studies in Crl:CD®(SD)BR rats, groups of 50 animals/sex/group were administered 0.0, 0.8, 3.0, 12.3, and 62.0 mg/kg-day chlorothalonil via their feed for two years (Spencer-Briggs,1995b, as cited in CDPR, 1997b). Epithelial hyperplasia and hyperkeratosis of the non-glandular forestomach were increased in both sexes at all dose levels. Squamous cell papillomas and carcinomas of the forestomach were observed in males (0/50, 0/50, 0/50, 0/50, and 3/50) with a significant trend (p < 0.01) and females (0/50, 0/50, 0/50, 2/50, and 1/50),
but no statistically significant increases in the incidence of treatment-related tumors were observed.

In consideration of the studies identified above, the most suitable carcinogenicity data for human cancer potency assessments come from the longer-term studies conducted in Fischer 344 rats by the IRDC (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005) and by IRDC/EPL (Wilson and Killeen, 1989). This is based on i) observations that rats appear to be more sensitive to the carcinogenic effects of chlorothalonil than mice; ii) the exposure durations of the IRDC and IRDC/EPL Fischer 344 rat studies were greater than the other available long-term rat studies (i.e., greater than two years); and iii) chlorothalonil was a more potent carcinogen in the IRDC and IRDC/EPL Fischer 344 rat studies than in the other available long-term rat studies. The dose response data for each study are presented in Tables 2 and 3 below.
Table 2. Tumor incidence in Fischer 344 rats administered chlorothalonil via feed for 27 months (males) and 30 months (females) (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005).

<table>
<thead>
<tr>
<th>Sex, strain, species</th>
<th>Tumor site and type</th>
<th>Average daily dose(^a) (mg/kg-day)</th>
<th>Tumor incidence(^b)</th>
<th>Statistical significance(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0</td>
<td>0/60</td>
<td>p &lt; 0.05(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1/60</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>1/60</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>175</td>
<td>3/60</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0</td>
<td>0/60</td>
<td>p &lt; 0.0001(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>7/60</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>7/58</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>175</td>
<td>18/60</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Female F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0</td>
<td>0/60</td>
<td>p &lt; 0.001(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>1/60</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>2/60</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>175</td>
<td>7/60</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0</td>
<td>0/60</td>
<td>p &lt; 0.0001(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>4/60</td>
<td>p = 0.059</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>10/59</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>175</td>
<td>23/60</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

\(^a\) As reported by CDPR (2005) and U.S. EPA (1999)  
\(^b\) As reported by CDPR (2005)  
\(^c\) Results of pairwise comparison using Fisher's Exact Test. NS is not significant.  
\(^d\) Exact trend test p-values.
Table 3. Tumor incidence in Fischer 344 rats administered chlorothalonil via feed for 23-26 months (males) and 29 months (females) (Wilson and Killeen, 1989).

<table>
<thead>
<tr>
<th>Sex, strain, species</th>
<th>Tumor site and type</th>
<th>Average daily dose(^a) (mg/kg-day)</th>
<th>Tumor incidence(^b)</th>
<th>Statistical significance(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male F344/N rats</td>
<td>Forestomach papilloma</td>
<td>0</td>
<td>0/55</td>
<td>(p &lt; 0.01)(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>2/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>182</td>
<td>5/55</td>
<td>(p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0</td>
<td>1/55</td>
<td>(p &lt; 0.0001)(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>4/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>182</td>
<td>23/55</td>
<td>(p &lt; 0.0001)</td>
</tr>
<tr>
<td>Female F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0</td>
<td>1/55</td>
<td>(p &lt; 0.001)(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2/55</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>5/53</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>182</td>
<td>9/55</td>
<td>(p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0</td>
<td>0/55</td>
<td>(p &lt; 0.0001)(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0/54</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0/55</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>0/53</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>182</td>
<td>32/55</td>
<td>(p &lt; 0.0001)</td>
</tr>
</tbody>
</table>

\(^{a}\) Calculated from data reported in Wilson and Killeen (1989)

\(^{b}\) As reported by Wilson and Killeen (1989)

\(^{c}\) Results of pairwise comparison using Fisher’s Exact Test. NS is not significant.

\(^{d}\) Exact trend test p-values.
APPROACH TO DOSE-RESPONSE ANALYSIS

This section briefly reviews the genotoxicity data and other data relevant to possible mechanisms of chlorothalonil carcinogenicity for the purpose of determining the most appropriate approach to dose-response analysis.

Genotoxicity

Chlorothalonil has been tested for gene mutations, chromosomal effects, and DNA damage in a variety of assays. It was positive in some but not in others. For example, as summarized by IARC (1999) and CDPR (2005), chlorothalonil induced mutations in bacteria in Salmonella typhimurium TA102, in the presence of metabolic activation (i.e., addition of S9), but not in S. typhimurium TA98, TA100, TA1535, TA1537, or TA1538, or in Escherichia coli WP2 hcr. Chlorothalonil induced mutations in the yeast Aspergillus nidulans, and in L5178Y tk+/− mouse lymphoma cells in two of three independent experiments (IARC, 1999; CDPR, 2005). With regard to chromosomal effects, chlorothalonil induced sister chromatid exchanges (IARC, 1999; CDPR, 2005) and chromosomal aberrations (CA) (CDPR, 2005; Vigreux et al., 1998) in Chinese hamster ovary (CHO) cells, but did not induce CA or micronuclei (MN) in Chinese hamster lung V79 cells or Chinese hamster lung V79 cells or BALB/c 3T3 fibroblasts. Chlorothalonil induced CA in the bone marrow of male Chinese hamsters in one study, but did not induce CA or MN in other studies in male Chinese hamsters, rats, or mice (CDPR, 2005).

Evidence that chlorothalonil induces DNA damage includes studies showing that it increases levels of the oxidized DNA product 8-hydroxy-2′-deoxyguanosine (8-OH-2dG), which is a mutagenic adduct, in rat liver in vivo in a dose-dependent manner (Lodovici et al., 1997). Additional evidence comes from various in vitro and in vivo studies measuring DNA damage, as detected by the comet assay, in rodents and humans. In vitro, chlorothalonil increased DNA damage in human peripheral blood lymphocytes at doses that had no immediate effect on cell viability (Lebailly et al., 1997) and in CHO cells in a dose-dependent manner at doses that did not induce immediate or delayed cytotoxic effects (Vigreux et al., 1998; Godard et al., 1999). In a study of male farmers in which mononuclear leukocytes were evaluated before and after a single day of spraying various mixtures of pesticides, increased DNA damage was observed in farmers that sprayed mixtures containing chlorothalonil (Lebailly et al., 1998). This DNA damage was observed in the absence of cytotoxicity or other effects on hematologic parameters, and was attributed by Lebailly et al. (1998) to chlorothalonil exposure. No DNA damage was detected by the comet assay in male Sprague-Dawley rats exposed to chlorothalonil in vivo (Godard et al., 1999). Finally, in studies investigating whether chlorothalonil binds to DNA, a single in vitro study reported very low levels (1-3%) of binding of 14C-chlorothalonil (radiochemical purity 96%) to mammalian DNA (Rosanoff and Siegel, 1981, as cited in CDPR, 2005), while other studies did not detect any level of covalent binding with DNA (Savides et al., 1987 and Shah et al., 1987, as cited in CDPR, 2005).

Chlorothalonil may be genotoxic due to its ability to induce oxidative DNA damage, to form mutagenic thiol metabolites, to bind covalently with DNA, and other mechanisms yet to be identified. 8-OH-2dG adducts, which are one manifestation of oxidative DNA damage, can lead to the formation of single point mutations as a result of G:C to T:A
transversions if not repaired before DNA replication (Shibutani et al., 1991). 8-OH-2dG adducts can also result in formation of DNA strand breaks, as a result of incomplete base excision repair (Hashimoto et al., 2007). DNA strand breaks may manifest as chromosomal effects (e.g., CA, MN) and as DNA damage detected by the comet assay. Electrophilic thiol metabolites, such as those derived from chlorothalonil-glutathione conjugates, have the potential to react directly with DNA and induce mutations (Anders and Dekant, 1998). The low level of covalent binding to DNA observed in vitro (Rosanoff and Siegel, 1981, as cited in CDPR, 2005) suggests the possibility that direct binding of chlorothalonil to DNA may occur to a limited extent in vivo.

Cell proliferation

Chlorothalonil induced cell proliferation in the forestomach and kidneys of male and female rats in long-term bioassays (e.g., Wilson, 1985 and Wilson, 1986, as reported in CDPR, 2005; Wilson and Killeen, 1989; Wilkinson and Killeen, 1996). Chlorothalonil has also been shown in shorter-term studies to induce sustained cell proliferation in rat forestomach and kidneys (U.S. EPA, 1999; Wilkinson and Killeen, 1996). For example, in 90-day dietary studies in male F344 rats, increased BrdU labeling was observed in the kidneys at day 7, day 28, and day 91 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999). Similarly, in 28-day dietary studies in male rats, increased proliferating cell nuclear antigen (PCNA) staining of proximal convoluted tubule epithelial cells and increased BrdU labeling of the forestomach were observed at day 7, day 14, day 21, and day 28 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999).

There may be multiple mechanisms by which chlorothalonil induces cell proliferation. One proposed mechanism involves the induction of cytotoxicity, accompanied by regenerative hyperplasia (a type of cell proliferation). Another involves activation of the erythroblastic leukemia viral (ErB-2) oncogene tyrosine kinase signal transduction pathway, which is independent of cytotoxicity. Regarding the first proposed mechanism, chlorothalonil-derived thiols have been shown to inhibit mitochondrial respiration, based on studies conducted with rat kidney subcellular fractions (Wilkinson and Killeen, 1996). Inhibition of mitochondrial respiration results in decreased formation of adenosine triphosphate (ATP), increased oxidative stress, and ultimately, cell death (Anders and Dekant, 1998). Wilkinson and Killeen (1996) proposed that cytotoxicity induced by chlorothalonil-derived thiols in the kidney leads to compensatory cell proliferation and hyperplasia that, if sustained, eventually results in tumor formation. Support for the second proposed mechanism comes from studies in LNCaP cells, a human prostate cancer cell line, in which chlorothalonil treatment increased ErbB-2 tyrosine kinase activity, mitogen-activated protein kinase (MAPK) phosphorylation, and cell proliferation (Tessier and Matsumura, 2001).

Histone protein binding

Chlorothalonil binds to cellular proteins, including histones in the nucleus. As reviewed by CDPR (2005), incubation of 14C-chlorothalonil with rat liver histones in vitro resulted in a significant degree of binding (i.e., >50% of total radioactivity). There are a number of adverse effects that might result from the binding of chlorothalonil to histone proteins which could be involved in the chemical’s carcinogenicity. These possibilities include
damage to key histone proteins involved in DNA replication and transcription processes, alteration of DNA structure (e.g., folding and packaging), DNA strand breaks, and alterations in global gene methylation level with resultant changes in gene expression patterns (Baccarelli and Bollati, 2009).

In summary, multiple mechanisms are likely to be involved in chlorothalonil’s carcinogenicity, including one or more involving genotoxicity. Therefore the default approach using a linearized multistage model is applied to derive a cancer potency estimate for each treatment-related tumor site observed in a given experiment. The default procedures are outlined in Title 27, California Code of Regulations, section 25703. A description of the methodology used is given in the Appendix.

DOSE-RESPONSE ASSESSMENT

Animal and human cancer potency estimates were derived for chlorothalonil by fitting the multistage model to the dose-response data from studies in male and female Fischer 344 rats conducted by the IRDC (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005) and by IRDC/EPL (Wilson and Killeen, 1989) (Tables 2 and 3).

The model fitting results in an animal cancer potency estimate, as described in the Appendix. Multiplying by the applicable interspecies scaling factor gives an estimate of human cancer potency for each treatment-related tumor site. Overall cancer potency estimates are based on the sum of potency estimates when multiple tumor types are observed within a given experiment. This calculation is performed using a Monte Carlo approach to statistically sum the potencies, as described in the Appendix. The results are summarized in Table 4 below.

The interspecies scaling factor is derived from the ratio of body weight in humans (assumed to be 70 kilograms [kg]) to the body weight of the experimental animals, as explained in the Appendix. For the Wilson (1985) study in male rats, an average body weight of 0.383 kg was calculated based on time-weighted average body weight data for control males. For the Wilson (1986) study in female rats, an average body weight of 0.240 kg was calculated based on time-weighted average body weight data reported for control females. For the Wilson and Killeen (1989) studies in male and female rats, the average body weights of 0.390 kg for males and 0.240 kg for females were calculated based on data reported for controls.

As shown in Table 4, the multisite human cancer potency derived from the Wilson and Killeen (1989) study in male rats of 0.026 (mg/kg-day)^{-1} is higher than that derived from either of the other three studies (i.e., Wilson (1985), Wilson (1986), and the female rat study of Wilson and Killeen (1989)). This value of 0.026 (mg/kg-day)^{-1} was selected as the human cancer potency estimate for chlorothalonil.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Sex, strain, species</th>
<th>Type of neoplasm</th>
<th>Animal cancer potency (mg/kg-day)$^{-1}$</th>
<th>Human cancer potency (mg/kg-day)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson (1985)</td>
<td>Male F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0.000554</td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0.00274</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multisite</td>
<td>0.00309</td>
<td>0.018</td>
</tr>
<tr>
<td>Wilson (1986)</td>
<td>Female F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0.000905</td>
<td>0.0060</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0.00309</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multisite</td>
<td>0.00357</td>
<td>0.024</td>
</tr>
<tr>
<td>Wilson and Killeen (1989)</td>
<td>Male F344/N rats</td>
<td>Forestomach papilloma</td>
<td>0.000989</td>
<td>0.0056</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0.00407</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multisite</td>
<td>0.00468</td>
<td>0.026</td>
</tr>
<tr>
<td>Wilson and Killeen (1989)</td>
<td>Female F344/N rats</td>
<td>Forestomach papilloma or carcinoma</td>
<td>0.00162</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal tubular epithelial adenoma or carcinoma</td>
<td>0.00115</td>
<td>0.0076</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multisite</td>
<td>0.00243</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**Bold** indicates the value selected as the basis for the NSRL.

**NO SIGNIFICANT RISK LEVEL**

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of $10^{-5}$. The human cancer potency estimate of 0.026 (mg/kg-day)$^{-1}$ for chlorothalonil, based on the data from male rats in the Wilson and Killeen (1989) studies, was used to calculate the NSRL for this chemical. The value of 27 μg/day was derived as shown below.
NSRL = \frac{10^{-5} \times 70 \text{ kg}}{0.026 (\text{mg/kg - day})^{\text{m}}} \times 1000 \text{ µg / mg} = 27 \text{ µg / day}
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California Department of Food and Agriculture (CDFA, 1988). Chlorothalonil (Bravo). Division of Pest Management, Environmental Protection and Worker Safety. California Department of Food and Agriculture. California Environmental Protection Agency, Sacramento, CA.


farmers measured using the alkaline comet assay: Modifications of DNA damage levels after a one-day field spraying period with selected pesticides. Cancer Epidemiol Biomarkers Prev 7:929-940.


APPENDIX: METHODOLOGY USED TO DERIVE THE NSRL FOR CHLOROTHALONIL

Procedures for the development of Proposition 65 NSRLs are described in regulation in Title 27, California Code of Regulations, Sections 25701 and 25703. Consistent with these procedures, the specific methods used to derive the NSRL for chlorothalonil are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

*Multistage polynomial model*

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (California Department of Health Services [CDHS], 1985; U.S. Environmental Protection Agency [U.S. EPA], 2002; Anderson et al., 1983):

\[
p(d) = 1 - \exp\left[-\left(q_0 + q_1d + q_2d^2 + \cdots + q_id^i\right)\right]
\]

with constraints, \(q_i \geq 0\) for all \(i\). The \(q_i\) are parameters of the model, which are taken to be constants and are estimated from the data. With four dose groups, as is the case with the Wilson (1985) and Wilson (1986) studies of chlorothalonil (as reported in CDPR, 2005), the default linearized multistage model defaults to three stages, or four parameters, \(q_0, q_1, q_2,\) and \(q_3\). With five dose groups, as is the case with the Wilson and Killeen (1989) studies of chlorothalonil, the default linearized multistage model defaults to four stages, or five parameters, \(q_0, q_1, q_2, q_3,\) and \(q_4\). Due to modeling constraints associated with the Wilson and Killeen (1989) female rat forestomach tumor data, a two-parameter model was used to fit these forestomach tumor data. The parameter \(q_0\) provides the basis for estimating the background lifetime probability of the tumor (i.e. 1 - \(\exp[-(q_0)]\)). The parameter \(q_1\) is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The upper 95% confidence bound on \(q_1\), estimated by maximum likelihood techniques, is referred to here as \(q_{1(UCB)}\). When the experiment duration is at least the natural lifespan of the animals, the parameter \(q_{1(UCB)}\) is taken as the animal cancer potency. When dose is expressed in units of mg/kg-day, the parameters \(q_1\) and \(q_{1(UCB)}\) are given in units of (mg/kg-day)^{-1}. Details of the estimation procedure are given in Crump (1984) and Crump et al. (1977).

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson et al., 1983; U.S. EPA, 2002) as “extra risk”, and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

*Multisite Procedure*

For carcinogens that induce tumors at multiple sites and/or with different cell types in a particular species and sex, the animal cancer potency is derived by probabilistically summing the potencies from the different sites and/or cell types. This is a way of taking...
into account the multisite carcinogenicity and provides a basis for estimating the cumulative risk of carcinogen treatment-related tumors.

The linear term ($q_1$) of the multistage model above is first estimated based on the dose-response data for each of the treatment-related tumor sites. Statistical distributions, rather than point estimates, are generated at each site for the linear term ($q_1$). The distributions of $q_1$ for each of the treatment-related tumor sites are then statistically summed using a Monte Carlo approach and assuming independence. The sum is created by adding the linear term for each tumor site, according to its distribution, through random sampling with 100,000 trials. The upper 95 percent confidence bound on the summed distribution is taken as the multisite animal cancer potency estimate.

**Adjustments for experiments of short duration**

To estimate potency in animals ($q_{animal}$) from experiments of duration $T_e$, rather than the natural life span of the animals ($T$), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{animal} = q_{1(UCB)} \cdot \left(\frac{T}{T_e}\right)^3$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting $T_e$ weeks in these rodents:

$$q_{animal} = q_{1(UCB)} \cdot \left(\frac{104}{T_e}\right)^3$$

Because the duration of the Wilson (1985), Wilson (1986), and Wilson and Killeen (1989) studies were each greater than 104 weeks, a correction factor to extrapolate to 104 weeks was not required and therefore $q_{animal} = q_{1(UCB)}$.

**Calculation of average daily dose**

For the studies by Wilson and Killeen (1989), the average daily dose of chlorothalonil was calculated based on the body weights of the animals, the amount of chlorothalonil added to the feed, and the feed consumption rates of the animals reported by Wilson and Killeen (1989). The average daily doses in the Wilson and Killeen (1989) studies were: 0, 2, 4, 16, and 182 mg/kg-day for male rats and female rats (calculated from data reported in Wilson and Killeen, 1989).

The average daily doses of chlorothalonil in the Wilson (1985) study in male rats and in the Wilson (1986) study in female rats were reported by CDPR (2005) and U.S. EPA (1999) to be: 0, 40, 80, and 175 mg/kg-day.
A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California carcinogen risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency ($q_{human}$) can be achieved by multiplying the animal potency ($q_{animal}$) by the ratio of human to animal body weights ($b_{wh}/b_{wa}$) raised to the one-third power when animal potency is expressed in units (mg/kg-day)$^{-1}$ (see Watanabe et al., 1992):

$$q_{human} = q_{animal} \cdot \left(\frac{b_{wh}}{b_{wa}}\right)^{1/3}$$

In the Wilson (1985), Wilson (1986), and Wilson and Killeen (1989) studies, average body weights were calculated based on time-weighted average body weight data for control animals. Average body weight was 0.383 kg for male rats in the Wilson (1985) study, 0.240 kg for female rats in the Wilson (1986) study, 0.390 kg for male rats in the Wilson and Killeen (1989) studies, and 0.240 kg for female rats in the Wilson and Killeen (1989) studies. The default human body weight is 70 kg. An example derivation of human cancer potency using the male rat multisite animal cancer potency of 0.00468 (mg/kg-day)$^{-1}$ from the Wilson and Killeen (1989) studies is shown below:

$$q_{human} = 0.00468 \text{ (mg/kg-day)}^{-1} \cdot \left(70 \text{ kg} / 0.390 \text{ kg}\right)^{1/3} = 0.026 \text{ (mg/kg-day)}^{-1}$$

A.3 Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk $R$, from exposure is:

$$I = \frac{R \times b_{wh}}{q_{human}}$$

where $b_{wh}$ is the human body weight, and $q_{human}$ is the human cancer potency estimate.

Daily intake levels associated with lifetime cancer risks above $10^{-5}$ exceed the NSRL for cancer under Proposition 65 (Title 27, California Code of Regulations, section 25703).

Thus for a 70 kg person, the NSRL is given by:

$$\text{NSRL} = \frac{10^{-5} \times 70 \text{ kg}}{q_{human}} \times 1000 \mu g / \text{mg}$$
APPENDIX REFERENCES


U.S. Environmental Protection Agency (U.S. EPA, 2002). *Health Assessment of 1,3-Butadiene*. National Center for Environmental Assessment, Washington D.C. EPA/600/P-98/001F.
