PROPOSED COSMETIC PESTICIDE BAN IN PROVINCE OF ONTARIO
SCIENTIFIC BASIS FOR BANNING BOTH SALE AND USE OF SYNTHETIC PESTICIDES

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SUMMARY

Human health and safety should be the primary consideration when drafting a law restricting the cosmetic use of pesticides. In this regard, the proposed provincial pesticide legislation, while necessary, has several aspects which, as a scientist, greatly concern me. The legislation is flawed in that it identifies a list of banned substances rather than specifying a list of permitted substances. This approach entails a potential for errors of omission and also, by its nature, encourages pesticide manufacturers to introduce new products to circumvent the ban. In addition, the proposed legislation allows for restricted use of certain products in conditions which may be broadly and subjectively applied, which undermines both its intent and effectiveness. For example, while initially excluded from the list of banned products, it is now being proposed that Roundup be permitted for restricted use. To permit this type of usage introduces a significant loophole wherein it would be impossible to monitor how, where and in what quantity this dangerous product is being used.

Further to this, I have compiled a summary of peer-reviewed research papers on Roundup/glyphosates conducted by non-industry funded scientists. This review focuses on human health effects and illustrates why a total ban on these substances is the only recourse for both human and environmental well-being. This review follows and I respectfully request that you take it into consideration.

INTRODUCTION

The literature does not support the concept that some pesticides are safer than others; it simply points to different health effects with different latency periods for the different classes. Given the wide range of commonly used home and garden products associated with health effects, our message to patients should focus on reduction of exposure to all pesticides rather than targeting specific pesticides or classes (1).

The pesticide Roundup and its active ingredient glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. It is used by both professional applicators and consumers. Glyphosate is one of the eight most common lawn-care chemicals in Canada (2). 5-7 million pounds of glyphosate are used annually in homeowner applications (3)

Commercial glyphosate-based formulations most commonly range from concentrates containing 41% glyphosate to 1% formulations marketed for domestic use. They generally consist of an aqueous mixture of the isopropylamine salt of glyphosate, a surfactant and other minor components including anti-foaming and colour agents, biocides and inorganic ions (4). The glyphosate component is used as five different salts and the surfactant components in commercial formulations vary in nature and concentration (4).

Glyphosate was formerly considered relatively non-toxic however there is now a considerable body of evidence for deleterious effects of Roundup, glyphosate and its adjuvants on a wide range of non-target species, including humans.

In 2003 the Danish Government announced unprecedented restrictions on glyphosate following analyses which demonstrated that it had been percolating through the soil and polluting the ground water at a rate 5-times that allowable for drinking water. Subsequently, another study confirmed that both glyphosate and its degradation product amino-methylphosphonic acid (AMPA) can leach through structured soils thereby posing a potential risk to the aquatic environment (5). More recently, an analytical method for glyphosate and AMPA based on liquid chromatography coupled to electrospray tandem mass spectrometry has been applied to water samples previously found to contain glyphosate (6). The glyphosate concentrations in the re-anaylzed samples were found to be 2 – 14 –fold higher than previously (6) suggesting that contamination of groundwater and other aquatic systems by glyphosate may be even greater than previously thought.

STUDIES AND RESULTS

Research on the effects of glyphosates on non-target species have consisted of *in vivo* and *in vitro* studies over a wide range of species, including humans.

1. *In vivo* studies, non-human species :

a. Freshwater microbial communities.

The effect of Roundup on phytoplankton and periphyton communities which are at the base of the aquatic food webs was assessed (7). In both the micro- nano-phytoplankton and periphytic assemblages there was a decrease in abundance with an increased ratio of dead to live individuals. In addition, there was a corresponding increase in the abundance of cyanobacteria in both communities, of 40- and 4.5- fold respectively (7). The observed changes were more consistent with a direct toxicological effect of glyphosate rather than an indirect effect mediated by phosphorous enrichment (7).

b. Molluscs

Native freshwater mussels are among the most imperiled faunal groups in the world and factors contributing to the decline likely include pesticides (8). The toxicity of several forms of glyphosate, its formulations and a surfactant (MON 0818) used in several glyphosate formulations was determined for early life stages of *Lampsillis siliquodea*, a freshwater mussel native to North America. Roundup, glyphosate alone, and MON 0818 were each highly toxic to *L. silquodea* glochidia and juveniles (8). MON 0818 was the most toxic of the compounds tested and its 48h median effective concentration for glochidia was the lowest reported for any aquatic organism tested to date. These results indicate that *L. siliquodea*, a representative of nearly 300 freshwater mussel taxa in North America, is among the most sensitive aquatic organisms to glyphosate-based chemicals and the surfactant MON 0818 (8).

c. Teleostan Fish.

1. Acetylcholinesterase (AChE) activity in brain and muscle, metabolic parameters in liver and muscle and hematological parameters were measured in *Leporinus obtusidens* after exposure to Roundup (9). The results indicate a decrease in AChE activity in the brains if fish exposed to all glyphosate concentrations. In addition, all metabolic parameters tested (hepatic and muscle glycogen, glucose, lactate, protein and ammonia levels) demonstrated either increases or decreases in the presence of glyphosate (9). Finally, there was a decrease in all hematological parameters tested (9). Similar results were found for the silver catfish (10).

2. Dose-dependent cytogenetic and DNA damage were observed in goldfish exposed to Roundup (11).

3. Short-term exposure of the Neotropical fish *Prochilodus lineatus* to sub-lethal concentrations of Roundup promoted antioxidant defenses, plasma glucose (a stress response) and histological liver alterations (12).

d. Amphibians.

1. Oxidative stress biomarkers, *in vivo* heart rate and contraction dynamics of the bullfrog *Lithobates catesbiana* tadpoles were evaluated after exposure to a sub-lethal dose of Roundup original (glyphosate, 41%) (13). The activities of superoxide dismutase and catalase were increased in the liver and decreased in muscle and oxidative damage to lipids increased in both tissues demonstrating generation of reactive oxygen species and oxidative stress (13). In addition, cardiac adjustments were observed *in vivo* and *in vitro*. The results demonstrate a high energetic cost and high oxidative stress in *L. catesbiana* in response to low and realistic concentrations of Roundup which could have an impact on tadpoles' performance and success, jeopardizing their survival and/or population establishment (13).

2. The impact of Roundup with and without chemical cues emitted by predatory newts on six species of North American amphibian larvae was examined (14). Roundup was lethal at concentrations 3 to 7-fold lower than previous estimates and in one of the species, the addition of predatory stress resulted in a 2-fold increase in the lethal effect of Roundup, suggesting synergism between predatory stress and pesticides (14).

3. Oviposition site selection by adult gray tree frogs in the presence of Roundup with and without the addition of predatory cues was examined (15). The frogs avoided oviposition in pools contaminated with Roundup and/or predatory cues providing evidence that Roundup at concentrations expected to be found in the field potentially alters oviposition site choice by amphibians (15) thus modifying their behaviour.

e. Mammals.

Male offspring of Wistar rats exposed to glyphosate during pregnancy and lactation displayed adverse reproductive effects including reduced sperm number and production and an increase in the percentage of abnormal sperms (17). These results suggest that *in utero* and lactational exposure of male rats to glyphosate may induce significant adverse effects on the reproductive system at puberty and during adulthood (17).

2. **Laboratory Studies**.

a. Developmental, Genetic, Epigenetic : Cell division in sea urchin embryos is a recognized model for cell-cycle studies. Recently sea urchin early development has been used for analyses of early steps in the cancerization process (19).

1. The effect of Roundup on cell-cycle regulation was studied using sea urchin first divisions following fertilization as a model for universal cell cycle regulation (20). The results showed (a). There was a dose-dependent delay in the first cell cleavage of embryos in response to non-lethal doses of Roundup in the presence of glyphosate and by glyphosate in the presence of sub threshold doses of Roundup, (b). There was a synergy between glyphosate and Roundup formulations in inducing this response , (c). The mechanism of this action was a delay in the activation of CDK1/cyclin B activation during the M-phase of the cell cycle and inhibition of global protein synthesis (20).

2. A study examined the ability of five different commercially available glyphosate formulations to interfere with cell-cycle regulation using the sea urchin model (21). All products tested induced cell-cycle dysfunction. The authors suggest high risk from glyphosate by inhalation for people in the vicinity of spraying which would expose them to 500- 4000- fold higher concentrations than those that induced cell-cycle adverse reactions in the test system.

3. Sea urchin hatching is a landmark of early development and is a transcription-dependent process (22). Glyphosate has been shown to adversely effect sea urchin development by impeding the hatching process (22). In addition, the surfactant component of commercial Roundup, polyoxyethylene amine, was highly toxic to the embryos when tested alone. The mechanism of inhibition of hatching was inhibition of global transcription. Dose-dependent inhibition by glyphosate of a cloned fragment of the sea urchin hatching enzyme was also observed (22).

4. Embryonic cells contain a functional DNA- damage checkpoint which is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism and for commitment to cell death by apoptosis in the case of failure to repair (19). Cancers result from dysfunction of DNA- damage checkpoints. The sea urchin model allows assessment of cancer risk from molecules before epidemiological evidence is available (19). Roundup was tested using this model and shown to activate the DNA-damage checkpoint of the first cell cycle of development (19) suggesting DNA-damaging and potential carcinogenic activity of this formulation.

b. Rat, *in vitro.*

1. The hepatic effects of Glyphosate-Biocarb in Wistar rats were examined (16). Sub-chronic treatment, even at the lowest dose tested, induced leakage of hepatic intracellular enzymes suggesting irreversible damage in hepatocytes. In addition, hepatic histological changes occurred including an increase in Kupffer cells and deposition of collagen fibres (16).

2. Bioenergetic functions of isolated rat liver mitochondria were used to test the toxicity of Roundup (18). Roundup formulation products demonstrated adverse effects on both aerobic respiration and electrolyte homeostasis.

c. Human cells *in vitro*.

1. Time – and concentration – dependent toxicity of glyphosate has been demonstrated in human placental JEG3 cells at concentrations lower than those found with agricultural use (23). In addition, the herbicide disrupts aromatase activity and mRNA levels. Since aromatase is essential for estrogen synthesis, it is concluded that glyphosate has endocrine-disruptive potential in human cells. Finally, as in *in vitro* and *in vivo* studies with other species, Roundup was always more toxic than its active ingredient glyphosate suggesting that the presence of Roundup adjuvants enhance glyphosate bioavailability and/or bioaccumulation (23).

   In another study, the toxicity and endocrine disruption potential of Roundup was examined using human embryonic 293, placental-derived JEG3, normal human placental and equine testis cells (24). Roundup displayed lethal effects on the cells and the embryonic cells were 2- 4-fold more sensitive than the placental cells. As in other studies, Roundup was more effective than glyphosate alone suggesting a synergistic effect by the adjuvants in Roundup. At non-lethal doses, Roundup was an aromatase inhibitor in cell lines, regardless of tissues or species (human, equine) (24). Similar results were observed in human peripheral blood mononuclear cells (25).

2. Glyphosate induced dose-dependent cytotoxic and genotoxic effects in both normal and human and human fibrosarcoma cells (26).

3. Gene expression is altered in MCF-7 cells by endocrine disruptors (a variety of chemicals that mimic steroid hormones or interact with their receptors or co-factors). *In vitro* DNA microarray analysis of glyphosate was carried out to evaluate its capacity to alter the expression of a variety of genes in human cells (27). A group of genes found to be dysregulated were further examined by real-time PCR to corroborate their altered states of expression (27).

There have been several epidemiological and some case-control studies which implicate deleterious effects of Roundup, glyphosates or glyphosate adjuvants in human health.

1. Two case-controlled studies one on Non Hodgkin's lymphoma (NHL) alone and one on hairy-cell leukemia, a rare form of NHL showed a dose-response association with exposure to glyphosate (28).

2. Adverse neurobehavioural developmental effects and increased risk for congenital anomalies in children of pesticide applicators have been associated with parental glyphosate exposure (29).

3. Amongst women in the Ontario farm population, preconception exposure to glyphosate among other pesticides is associated with elevated risk of late spontaneous abortion (30).

4. There is also evidence from an Ontario study that there is a decrease in fecundability, as measured by time to pregnancy, when women were engaged in pesticide activities with specific pesticide active ingredients, including glyphosate (31).

5. Associations between glyphosate exposure and cancer incidence was evaluated in a large prospective cohort study of 57,311 licensed pesticide applicators in two U.S. States (the Agricultural Health Study). Results suggested an association between glyphosate exposure and multiple myeloma incidence (32).

IMPLICATIONS AND CONCLUSIONS

Roundup, glyphosate and its adjuvants demonstrate a wide variety of adverse effects on the health and functioning of numerous species, from the base of the aquatic food web to humans.

Implications for Human Health :

Roundup, glyphosate and its adjuvants have been shown to adversely effect several aspects of health, including cell-cycle regulation and cell-cycle DNA-damage checkpoints, an early stage in transformation of stem cells from normal to cancer. In addition, Roundup demonstrates toxic and hormone disruptor activity in human embryonic and placental cells, the effects of which are amplified with time, suggesting that exposure may effect human reproduction and fetal development. Glyphosate exposure has also been associated with cancer (multiple myeloma) incidence. Synergistic effects of glyphosate and the adjuvants in Roundup appear to further potentiate its adverse effects in several of the systems tested. Epidemiological studies also demonstrate that exposure to glyphosate is associated with spontaneous late-term abortions and adverse neurologic and neurobehavioural effects in children of glyphosate applicators.

Finally, genetic mapping has allowed the identification of gene polymorphisms in individuals that are associated with impaired metabolization of xenobiotics, biologically active substances that are foreign to the body which include drugs and pesticides. Inheritance of unfavourable "poor metabolizer" versions of the genes of the three main systems in pesticide detoxification has been shown to cause increased activation or reduced detoxification and elimination of environmental mutagens such as pesticides. For example, it has been shown (35) that 2,4-D use, both during pregnancy and childhood showed a consistent interaction with "poor metabolizer" genes and was associated with a 2-fold increase in leukemia incidence. It is not unreasonable to expect that similar associations for other pesticides will be found.

Recent biomonitoring studies in the U.S., particularly those assessing exposure in children, suggest that there may be widespread, low-level exposure to some commonly used home and garden pesticides and their degradation products (34). The data suggest that Roundup exposure may effect human reproduction and fetal development. In addition, the toxic and hormonal impact of chemical mixtures in Roundup formulations (24) and actual levels of glyphosate in the field (6) appear to have been underestimated. As previously suggested for 2,4-D exposure (35), given its widespread use, even if Roundup is associated with only modest increases in human health risk, it is of concern given the potential for population exposure.

The error of omission of Roundup from the list of banned substances in the proposed provincial legislation combined with the very large body of evidence for adverse human health effects and damage to the environment of pesticides in general illustrates that

nothing short of a total ban on the sale and use of synthetic lawn and garden pesticides is acceptable.

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