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The High Cost of Pesticides: Human and Animal Diseases

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Abstract

A significant degradation in the health of wild animals in Montana has been recorded over the past two decades. We surmise that the health issues are related to pesticide exposure. We present some of the evidence of the deterioration of the health in wildlife, which we used to inspire investigations on human health in the US population. While the animals’ exposure is through food, water and air, we believe that human exposure is predominantly through food, as the majority of the population does not reside near agricultural fields and forests.

We have obtained US government data on pesticide usage and on human disease patterns over time from the 1998-2010 hospital discharge data. Since glyphosate is by far the most widely used herbicide, we believe it to be a major source of contamination for humans. Correlations between glyphosate usage and specific health issues, along with the known toxicology profile of glyphosate obtained from the literature, reflect a plausible causal relationship.

Because much of the wildlife data is from deer fawns, most of the human data presented here involve newborn infants, but we also present some data for children 0-15 years old and for the full population (except newborn). We found many diseases and conditions whose hospital discharge rates match remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops. These include head and face anomalies (R=0.95), newborn eye disorders, newborn blood disorders (R=0.92), newborn skin disorders (R=0.96), lymph disorders in children 0-15 (R=0.86) and in the general population except newborn (R=0.89), congenital heart conditions in newborns (R=0.98), enlarged right ventricle in all age groups except newborn (R=0.96), newborn lung problems (R=0.95), pulmonary bleeding and edema for all age groups except newborn (R=0.97), liver cancer for all age groups except newborn (R=0.93), newborn metabolic disorders (R=0.95) and newborn genitourinary disorders (R=0.96).

Keywords: Glyphosate; Brachygnathia; Hypothyroidism; Congenital heart defects; Thymus; Lymphedema; Hepatic carcinoma; Hypospadias; Genitourinary disorders

Introduction

One of the promises assured with genetically engineered (GE) herbicide-resistant crops was that they would require many fewer pesticides, providing a more sustainable agricultural option. Several GE crops, including cotton, canola, corn, soy, sugar beets and alfalfa, are engineered to withstand direct application of glyphosate, the active ingredient in the pervasive herbicide, Roundup. As a result of the widespread acceptance of GE crops, the increasing practice of using glyphosate for pre-harvest dry-down in grains and legumes, along with the emergence of glyphosate-resistant weeds, the use of glyphosate has skyrocketed since 1996 [1-3].

With the exception of glyphosate, pesticide use on crops was indeed reduced for the first 5 or 6 years after the introduction of these GE crops. Then something happened after about 2002, resulting in a steep increase in glyphosate and 2,4-D applications on corn, soy and potato, along with an increase in dicamba on wheat. This coincides with a steep increase in the number of confirmed cases of glyphosate-resistant weeds [1] as shown in Figure 1.

The active ingredient in the pesticides is usually an acid. To make the pesticides more water soluble and therefore easier to package and distribute, they are chemically altered into a salt or ester formulation. Various salt formulations include isopropylamine, diammonium, monoaammonium, or potassium as the cation. Adjuvants are increasingly added to enhance the efficacy of the herbicides, particularly with the advent of the glyphosate-resistant weeds. One adjuvant is oxalic acid or an oxalate salt (potassium oxalate, e.g.) added to the stable salt formulations. For example, a 2006 patent by Xue et al. [4] discloses pesticide compositions, especially storage-stable herbicidal concentrates, containing oxalic acid and glyphosate that allegedly exhibit enhanced efficacy due to the addition of oxalic acid to glyphosate.

Figure 1: Glyphosate applications to corn, soy and along with the advent of glyphosate-resistant weeds.

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malformations and other diseases in humans follow the trends in D, chlorothalonil and glyphosate use. We also show that congenital malformations and wildlife diseases follow the trends for dicamba, 2,4-

data on selected crops from the USDA. We show that congenital hospital discharge data. Finally, we obtained pesticide application in children 0-15, and all age groups (except newborn) from the US malformations and diseases in newborn infants, along with diseases

In addition, we obtained corresponding data for human congenital in Montana wildlife for the past 19 years. In this paper, we present and again when nearly all of the formulations used were salts.

formulations. In the pesticide data shown in Figures 2-7, arrows have 2006) nearly all of the applications for a given herbicide were salt total amount of pesticides applied, but within a couple of years (by 2002) 1998 -- -- -- -- -- --

One of us (Hoy) has been documenting congenital malformations in Montana wildlife for the past 19 years. In this paper, we present documentation of wildlife deformities and evidence of organ damage. In addition, we obtained corresponding data for human congenital malformations and diseases in newborn infants, along with diseases in children 0-15, and all age groups (except newborn) from the US hospital discharge data. Finally, we obtained pesticide application data on selected crops from the USDA. We show that congenital malformations and wildlife diseases follow the trends for dicamba, 2,4-

The hypothesis presented for its effect is that it increases cell membrane permeability, suppresses oxidative burst, or increases expression of hydroxyproline-rich glycoproteins. However, it is also likely that it inhibits the breakdown of glyphosate, since oxalate inhibits the breakdown of glyoxylate, which is a disintegration product of glyphosate [5]. This would lead to an accumulation of glyoxylate, a strong glycating agent that would damage membrane fatty acids, explaining the increase in membrane permeability [6]. This patent also discloses that a variety of surfactants, including amines, amine oxides and quaternary ammonium compounds, can be used in combination with oxalic acid for pesticide compositions.

Manufacturers of pesticides do not disclose the adjuvants and surfactants used in any of their products, claiming that they are trade secrets. Only the formulation of the active ingredient can be traced. Table 1 shows the various formulations for 2,4-D, dicamba and glyphosate use. We hypothesize that the primary exposure route for humans is through food, whereas the primary exposure for animals is not only food but also direct exposure through air and water. Some of these conditions show a steep increase at the same time that the switch to the salt formulations of the herbicides was made.

Table 1: Salt and Ester Formulations of Pesticides.

<table>
<thead>
<tr>
<th>Pesticide applications to Corn</th>
<th>Year first applied to crop</th>
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<tbody>
<tr>
<td>Year</td>
<td>Corn</td>
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<tr>
<td>2002</td>
<td>3.5% of glyphosate as salts; in 2003, 96% were salts (blue arrow)</td>
</tr>
<tr>
<td>Total 2,4-D (6,000 lbs)</td>
<td>Total dicamba (4,200 lbs)</td>
</tr>
<tr>
<td>2001</td>
<td>64</td>
</tr>
<tr>
<td>2006</td>
<td>2007</td>
</tr>
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Figure 2: Herbicide applications to corn. Scale varies for each as indicated in the legend. Glyphosate salts were first applied in 2002, almost exclusively applied by 2005. Dicamba salts were first applied in 1998, almost exclusively by 2005. EPTC and alachlor applications have steadily decreased, becoming negligible by 2002 (not shown). Atrazine applications to corn crops have remained approximately constant at about 52.5 million pounds (not also shown).

Data Collection Methods

Pesticides

The United States Department of Agriculture National Agricultural
Statistics Service (USDA:NASS) maintains a database of US crops. Every year they randomly select fields of certain crops and send surveys to the persons who manage those fields. Among other things, they ask what herbicides were used, the application rate and how many times it was applied. Surveys are only sent to the states that are the major producers of a given crop, usually accounting for about 90% of the total US acreage planted in that crop. They then perform a statistical analysis and report the total acreage planted, the Percentage of Acres Treated (PAT) with each herbicide for that crop and the application rate per acre per year. One can then calculate the total amount of an herbicide that was applied to that crop in the survey states for that year.

Data files containing the information for pesticide applications are available from 1990-2012 [7]. We extracted the data for glyphosate, 2,4-D, dicamba, chlorothalonil, ETPC (S-Ethyl Dipropylthiocarbamate), atrazine and alachlor applications to corn, soy, potato and wheat crops. Sampling errors for the pesticide application data were less than 5% for most of the pesticides over most of the time period examined. Sampling errors (reported as standard errors) are small (<5%) in both the PAT and the application rate if the PAT is greater than 50%. Sampling errors are 5-10% if the PAT is between 10-50%, while the sampling errors are 10-100% if the PAT is <10%. The PAT for chlorothalonil on potato has exceeded 50% since 1994. The PAT for glyphosate on soy has exceeded 50% since 1998. The PAT for glyphosate on corn has exceeded 50% since 2006. The PAT for 2,4-D on spring wheat exceeds 50% over the entire data range. The PAT for dicamba ranges between 20% and 55% for spring wheat. By 2006, the PAT for glyphosate on spring wheat was 31%, durum wheat was 48% and winter wheat was 15%. Details on the PAT with glyphosate on corn and soy can be found in [3].

Wildlife

Wild ruminates were the primary animals studied. The study area where white-tailed deer (Odocoileus virginianus) were examined is Ravalli County, in the far western portion of Montana. The north flowing Bitterroot River forms the Bitterroot Valley (BV) located...
mandibular brachygnathia (MB, or overbite), the distance between the lower incisors was measured in millimeters on all examined white-tailed deer fawns each year from spring of 1995 through spring of 2014.

The heart and lungs were examined on all necropsied deer, both adults and fawns. The severity of the enlargement of the right ventricle of the heart on each deer was designated with a number beginning with 0 for hearts with no enlargement (normal) to 3 for a severely enlarged right ventricle. Similarly, the inflammation of the lungs was recorded with 0 being normal, and 1 through 3 designating the severity of inflammation, and 4 to designate that the animal had died of a hemorrhage in the lungs.

To quantify the genital hypoplasia on male fawns, a measurement from the body wall to the tip of the penis sheath and a measurement of the scrotum from the body wall to the lowest point on the scrotum were taken. To document the misalignment of the hemiscrota, the length from anterior to posterior and the width from side to side of the scrotum were measured. The length of the testes was measured for comparison with the hemiscrota length. Whether the testes were in the hemiscrota or partly or completely ectopic was recorded.

In addition, a study of genital hypoplasia and shortened urogenital distance in male eastern fox squirrel (Sciurus niger) populations in Northern Ravalli County, MT is currently in progress. There appear to be significant numbers of similar reproductive defects on examined males of several other Western Montana rodent species, including deer mouse (Peromyscus maniculatus), house mouse (Mus musculus), red squirrel (Tamiasciurus hudsonicus), northern flying squirrel (Glaucomys sabrinus), yellow pine chipmunk (Eutamias dorsalis) and yellow-bellied marmot (Marmota flaviventris).

Humans

Hospital discharge data, containing diagnoses collected from hundreds of hospitals by the United States Centers for Disease Control and Prevention (CDC) can give a snapshot of disease trends over time. These data are available for free download from the Web. Raw data files were available from 1998 through 2010. We downloaded the files and documentation from the CDC website. Each data file contains thousands of discharge records collected from hospitals using a statistically random sampling procedure [9]. The records contain information about the age, sex, race, geographic location and diagnoses for each discharge. The diagnoses are recorded by the International Classification of Diseases, Ninth Revision (ICD-9) codes. Up to seven diagnostic codes can be recorded for each discharge, with the first listed being the primary reason for hospital admission. We included in the set for any particular ICD-9 code any event which mentioned that code as one of the diagnostic codes for the event; i.e., we did not treat the first-mentioned code in any special way.

A computer program was written to query the data file for specific ICD codes for each year. We were interested in disease trends for three distinct age groups: (1) infants (<6 days old), (2) children (6–15 years old), and (3) all ages except infant (6 days–100 years old). A rate of increase, as an estimate of prevalence, over time for each particular diagnosis was obtained as follows:

\[ \hat{a} = a \times T / (t \times P) \]

where \( \hat{a} \) is the normalized hospital discharge rate for a disease in a year; \( a \) is the total number of the hospital discharge records of the disease in the year computed from the raw files; \( T \) represents the total number of hospital discharges in that year in the US; \( t \) is the total number of
hospital discharge records in the sampled hospitals in that same year, which is computed from the raw files; and P is the total population in the US for that year. Population estimates were obtained from the CDC. For the newborn data, we assumed that T/P = 1, i.e. the number of hospital discharges for newborns was equal to the population of newborns.

Using the information in the CDC documentation, we calculated some of the standard errors in these data. The standard errors for the general population were all less than 10%. The largest standard errors were for the acquired hypothyroidism in children, which ranged between 22%-37%. Standard errors for newborns could not be calculated because they were not included in the CDC tables.

Results

US trends in pesticide usage

With the exception of glyphosate, pesticide use on crops decreased for the first 5 or 6 years after the introduction of GE crops in 1996. Survey data from the US Department of Agriculture [7] show that the use of 2,4-D and dicamba on corn steadily decreased starting about 1996 as shown in Figure 2. Applications of EPTC and Alachlor also decreased, but the use of Atrazine has remained constant. The largest use of 2,4-D on soy also started decreasing in 1996 as shown in Figure 3. In the meantime, glyphosate was being promoted as a pre-harvest treatment to grain, dried pea and bean, and potato crops for more even ripening, dry-down and pre-harvest weed control [10]. The use of 2,4-D and dicamba on wheat decreased, being replaced by glyphosate starting in early to mid 1990s (Figures 4-6). With the exception of fungicides used for potato blight, pesticide applications to potatoes were also decreasing (Figure 7).

After about 2002, there was a steep increase in glyphosate and 2,4-D applications on all of these crops, along with an increase in dicamba on wheat. This coincides with a steep increase in the number of confirmed cases of glyphosate-resistant weeds as shown in Figure 1.

As seen in the Figures, not all crop data were reported for all years. Data for glyphosate applications to corn, soy and wheat were interpolated as outlined in [3] and the results are shown in Figure 8.

Pesticide use in the region of interest

Prior to 1994, there was extensive use of multiple herbicides and other pesticides, especially 2,4-D and dicamba, on wheat, potato and other crops in Idaho, Washington, Oregon and other states upwind of Western Montana as shown in Figures 7 and 8. Glyphosate was also being used prior to 1994, and its use has increased significantly since 1996. The formulation for glyphosate and other commonly used herbicides applied during the growing seasons in 2006 and 2007 and since was changed to salt formulations [4] (we hypothesize that oxalic acid was introduced with these salts as an adjuvant, but this can not be confirmed).

In addition to glyphosate, 2,4-D and Dicamba as shown in Figure 8, other pesticides were widely used in Western United States prior to 1994, including picloram, atrazine and several organochlorine herbicides. Multiple fungicides were used on over 500,000 acres of potato fields in Idaho, Washington and Oregon. Many types of insecticides were also used in Western Montana and states upwind long before 1994. Even with this extensive exposure to multiple wind drift and locally applied pesticides, almost no birth defects were observed or reported on developing young in Western Montana until 1995. An epidemic of multiple birth defects began being observed on many individuals of domestic and wild animals born that spring [10,11], with a significant increase in many of the birth defects over the study period, despite substantial annual variability.

Development and health issues in wild and domestic animals

In the case of the ungulates, we tabulated frequencies of multiple developmental defects as discussed in the Methods section, and noted a general pattern consisting of a high rate of disease early in the study period, a gradual decline until around 2006 and then a generally rising trend subsequently. This is consistent with the trends in pesticide use shown in Figures 2-8. We hypothesize that chlorothalonil on potatoes, along with dicamba and 2,4-D on the other crops, may contribute significantly to the early disease patterns in wildlife, whereas glyphosate is a major factor in the later rise in observed frequency.

We sought human data on disease trends in the hospital discharge data that would correspond as much as possible with the observed defects in the wild animals. This was not always easy, as jaw malocclusion is not reported explicitly in the database, nor is genital malformations. However, there are several malformations of the lower face that are tracked, such as dent facial anomalies (ICD 526), diseases of the jaws (ICD 527), diseases of the salivary glands (ICD 527) and diseases of the oral soft tissues (ICD 528), whose trends can be compared with those observed in the animals with jaw malformations. The plot we obtained for human urogenital disorders encompasses hydronephrosis (watery fluid around one or both testicles, ICD 778.6); hypospadias (ICD 752.6); and hydronephrosis -- obstruction of urine flow (ICD 591), and other disorders of the kidney and ureter (ICD 593). Thymus involution and dysfunction, notable in postmortem examination of the wild animals, is not normally indicated in ICD-9. Although a code exists for diseases of the thymus (254.8), it is almost never used (only 2 cases among the infant and newborn data in our data set). However, T-lymphocytes mature within the thymus gland, so its impairment can be reasonably linked to immune system disorders. In most other cases, such as the organ tumors, eye deformities, skin disorders, liver cancer and metabolic issues documented on wild and domestic animals, a more direct comparison was possible.

Our results are illustrated in Figures 9-32, and are discussed below in more detail. In addition to plots where we superimpose time trends for human data or wild animal data with pesticide usage, we also provide photos taken of a variety of wild and domestic animals.
exhibiting pathologies (Figures 9, 12, 14, 16, 18, 20, 24, 26 and 27).

Congenital head and facial malformations

Brachygnathia superior (BS), the underdevelopment of the upper facial bones of ungulate species, has been photographed in countries around the world and posted on the Internet, usually labeled as under bite. In Montana and throughout the United States, wild and domestic ungulate species appear to have an extremely high prevalence of BS, including our main study species, white-tailed deer [11] as shown in the photos in Figure 9. The percentage of white-tailed deer with BS has increased significantly in this region since 1995, as shown in Figure 10. Figure 10 also shows the prevalence of head, face and musculoskeletal anomalies in newborn infants superimposed with glyphosate applications to wheat, corn and soy crops. The newborn data correlate with glyphosate usage with a Pearson correlation coefficient of $R=0.947$.

We also noticed that trends in hypothyroidism in children aged 0-15 were rising, and that these patterns aligned very well with the data on brachygnathia in wild animals, both exhibiting a sharp peak in 2007 (Figure 11) approximately coincident with the changeover to salt formulations in the herbicides. Congenital hypothyroidism is common, and it is linked to other congenital disorders, for example hearing loss [12] and renal and urinary tract disorders [13]. According to Kumar, et al. [13], “Congenital hypothyroidism is the most common congenital endocrine disorder, affecting 1 in 3000 to 4000 newborns. Its incidence has increased 138% from 1978 to 2005 in New York State and 73% in the US from 1987 to 2002.”

Disorders of the eyes

Figures 12 and 13 depict eye disorders in wild animals and humans respectively. Figure 12 illustrates several cases of various eye deformities in black-billed magpies (Pica hudsonia), great horned owls (Bubo virginianus), a western toad (Bufo boreas), a pygmy goat, and severe blepharitis on a white-tailed deer fawn that were documented by Hoy.

Figure 13 shows the time trends of congenital disorders of the eye exhibiting.
in newborns, superimposed with pesticide applications to wheat, corn, soy and potato crops. Eye disorders include congenital anomalies of eyelids lacrimal system and orbit (ICD 743.6); disorders of conjunctiva (ICD 372); other disorders of the eye (ICD 379).

Figure 13: Hospital discharge rate for congenital eye disorders superimposed with pesticide applications to wheat, corn, soy and potato crops. Eye disorders include: congenital anomalies of eyelids lacrimal system and orbit (ICD 743.6); disorders of conjunctiva (ICD 372); other disorders of the eye (ICD 379).

There is no data in the hospital discharge records on the thymus. However, since hematopoietic progenitor cells enter the thymus from the blood and then multiply to generate a large population of T-cells, there should be some relationship between thymus impairment and diseases of the blood, especially white blood cells. We combined the following newborn blood disorders: transient neonatal thrombocytopenia (ICD 776.1), cutaneous hemorrhage of fetus or newborn (ICD 772.6), diseases of white blood cells (ICD 288) and nonspecific findings on examination of blood (ICD 790), to form the plot shown in Figure 15. While these conditions are only indirectly related to thymus problems, the trend is well matched to the rise in glyphosate usage on crops (R=0.92; p<8.2E-5). Lymphatic disorders are also rising in the human population, as discussed later in this section.

Newborn skin disorders

In recent years, observation of skin disorders, rash, blistering and

Figure 14: Newborn white-tailed deer thymus conditions. A and B. Normal thymus color and shape. C and D. Thymus with red spots throughout. E. Odd shaped, mostly red thymus. F. Undersized thymus, red throughout.

Figure 16: Skin disorders on wild and domestic mammals. A. Large blisters at the base of the right ear on a male WTD fawn, born 2013. B. Hair loss on shoulders, sides and hind legs of a female WTD fawn after exposure to environmental toxins, born 2003. C. Male WTD fawn inner ear skin with chemical blistering, born 2010. D. Young male eastern fox squirrel’s left ear showing severe chemical skin blisters in 2005. E. Adult female dog with chemical blisters, summer 2013. F. Adult female WTD with multiple skin growths in May, 2010.
skin tumors have been increasing on birds and wild and domestic mammals (Figure 16). We consequently examined data for skin disorders on humans. Figure 17 shows newborn skin disorders and skin disorders for the general population superimposed with glyphosate applications to wheat, corn and soy crops. The newborn skin disorders include: atopic dermatitis (ICD 691); pilonidal cyst (ICD 685); erythema and urticarial (ICD 778.8); vascular hamartomas (benign tumors) (ICD 757.32); pigment anomalies (ICD 757.33); unspecified deformities of hair, skin and nails (ICD 757.9); and meconium staining (ICD 779.84). The Pearson correlation coefficient with glyphosate usage is R=0.963. Skin disorders for the general population include: rash, swelling and changes in skin tone and texture (ICD 782); eczema (ICD 692); and psoriasis (ICD 696). The Pearson correlation coefficient with glyphosate usage is R=0.899.

Lymphatic disorders in the non-newborn populations

The thymus regulates the immune system; therefore, any problems with the thymus will result in a compromised immune system. The human lymphatic disorders, in particular, dramatically increased in 2007 at the same time that almost all of the glyphosate was being used as a salt formulation.

In conjunction with the increase in birth defects after spring of 1995, necropsied wildlife and domestic ruminants of all ages had various degrees of dilation of the lymphatic vessels on the surface of their hearts. The lymphatic vessels on hearts, especially of newborns, were more severely affected beginning in 2007, as illustrated by the last two photos of fawn hearts shown in Figure 18. Data for humans were examined for similar effects on the lymphatic system. The increase in lymphatic disorders among humans is not restricted to the infant population. Figure 19 shows the hospital discharge rate for children aged 0-15 with lymphatic disorders, superimposed with glyphosate applications to wheat, corn, and soy crops. The disorders include: lymphedema (ICD 457), lymphocytosis (ICD 288.6), and Castelman’s disease (angiofollicular lymph node hyperplasia) (ICD 202). The Pearson correlation coefficient is R=0.861. Figure 19 also shows the hospital discharge rate of these same lymphatic disorders over the full age range (except newborn). The correlation coefficient between this and glyphosate applications to wheat, corn, and soy crops is R=0.885.

Diseases and malformations of the heart and lung

On necropsied deer of all ages, the prevalence and severity of enlarged right heart ventricle (Figure 18) and emphysema-like symptoms on lungs (Figure 20) were high in 1998 and 1999, and then decreased until 2005, when these unusual conditions of the heart and lung increased dramatically, as shown graphically in Figure 21. Again, the increase after 2005 is approximately coincident with the switch-over to salt formulations in the herbicides.
Liver disease

An increasing number of mammals and birds have been observed with liver tumors, enlarged liver or liver involution. Figure 24 shows several examples of liver disease in wildlife, including tumor-like growths in a wolf (*Canis lupus*), a domestic goat, a fledgling Rock

Figure 23 shows newborn lung conditions superimposed with pulmonary bleeding and edema for all ages (except newborn), and with glyphosate usage on wheat, corn, and soy crops. The Pearson correlation coefficient between the newborn lung conditions and glyphosate applications is R=0.949, and for all ages (except newborn) R= 0.971.

Liver disease

An increasing number of mammals and birds have been observed with liver tumors, enlarged liver or liver involution. Figure 24 shows several examples of liver disease in wildlife, including tumor-like growths in a wolf (*Canis lupus*), a domestic goat, a fledgling Rock

We compared this trend with human data in Figure 22. Both newborn data for congenital heart disorders and data for all ages (except newborn) on enlarged right ventricle show remarkable correspondence with glyphosate usage on core crops. The tabulated newborn heart conditions include: heart murmur (ICD 785.2); ostium secundum type atrial septal defect (ICD 745.5); patent ductus arteriosus (ICD 747.0); pulmonary artery anomalies (ICD 747.3); other congenital anomalies of circulatory system (ICD 747.8); other heart/circulatory conditions originating in the perinatal period (ICD 779.89); and bradycardia (ICD 779.81,427.89). The Pearson correlation coefficient between congenital heart defects and glyphosate applications is R=0.983, and for enlarged right ventricle it is R=0.955.

Figure 23 shows newborn lung conditions superimposed with pulmonary bleeding and edema for all ages (except newborn), and with glyphosate usage on wheat, corn, and soy crops. The Pearson correlation coefficient between the pulmonary disorders and glyphosate is R=0.9705. The newborn lung conditions include: asphyxia and hypoxemia (ICD 799); pulmonary artery anomalies (ICD 747.3); meconium passage during delivery (ICD 763.84); and other respiratory conditions of fetus and newborn (ICD 770). The Pearson correlation coefficient between the newborn lung conditions and glyphosate applications is R=0.9491.
29. Several of the reproductive malformations have not been well studied, especially misplacement forward of the inguinal lymph node and the left spermatic cord, resulting in misalignment of the testes and corresponding hemiscrota during fetal formation of the scrotal sac (Figure 26C, 26E and 26F). This easily observed reproductive malformation was first reported in a 2002 study of white-tailed deer [10]. It has become very high in prevalence in white-tailed deer (Figure 30), and appears to also be high in several Western Montana rodent species, especially the introduced eastern fox squirrel (Figure 27). In

Liver cancer in humans has also been increasing in frequency in the United States over the past two decades, with a shift towards relatively younger ages [14]. Similar trends are seen in China [15]. Figure 25 shows the hospital discharge rates of liver cancer in all ages (except newborn), alongside glyphosate usage on core crops. The Pearson correlation coefficient between the prevalence of liver cancer and glyphosate applications is R=0.9316.

**Congenital urogenital malformations**

Birth defects of the male reproductive organs (Figures 26 and 27) have become common on mammals in Montana and appear to be occurring in some wildlife populations over much of the US [16,17]. The decrease in penis sheath length, scrotum size and the change in testes position in Montana are depicted in Figures 28 and 29.
27 specimens of male eastern fox squirrel examined by Hoy from 2010 through 2014, 78% (21) had no hemiscrota formed and 7% (2) had one hemiscrota formed, all with ectopic testes, leaving only 15% (4) with a normal scrotum containing both testes. On 89%, (24) the penis sheath was less than half normal length.

Figures 28 and 29 show our data collected from WTD from 1995 to 2010 on penis sheath length (Figure 28) and testes position and scrotum length (Figure 29). In almost all years, fewer than half of the animals examined had a normal configuration. Notably, in 2006, 100% of the animals examined had ectopic testes, and more than 90% had a misaligned scrotum.

Figure 31 shows newborn genitourinary disorders compared to glyphosate applications to wheat, corn and soy crops. The human disorders include: hydrocele (watery fluid around the testicles) (ICD 778.6); hypospadias (ICD 752.6); hydronephrosis – obstruction of urine flow (ICD 591); and other disorders of the kidney and ureter (ICD 593). The Pearson correlation coefficient between genitourinary disorders and glyphosate applications is R=0.959.

Failure to thrive, observed on multiple species of wild newborns, is a recognized problem in livestock, and may well be related to human failure to thrive. For example, porcine periweaning failure-to-thrive syndrome (PFTS) is an increasingly recognized syndrome in the swine industry in North America [17]. It is characterized by anorexia developing within one week of weaning followed by lethargy and, in some cases, death.

We examined the data in human newborns for comparison and found that a number of metabolic disorders have been increasing in frequency in human newborns, as illustrated in Figure 32, in step with glyphosate applications to wheat, corn and soy crops. Included are: disorders of fluid electrolyte and acid/base balance (ICD 276); underweight, feeding problems and fetal malnutrition (ICD 783); disorders of mineral metabolism (ICD 275); slow fetal growth and fetal malnutrition (ICD 764); and other congenital anomalies of the
upper alimentary tract (ICD 750). The Pearson correlation coefficient between these two plots is R=0.949.

**Discussion**

One of us (Hoy) has been documenting health status of wild animals in the mountains of Western Montana for over forty years. She has noticed a significant degradation in health over the past two decades, mainly consistent with mineral deficiencies and thyroid hormone disruption, and she surmises that the health issues are related to exposure to various pesticides being applied to crops in close proximity to the animals’ habitat. Besides exposure from nearby applications, many pesticides have been shown to travel on fast-moving weather fronts to come down in rain or snow many hundreds of miles from the application site [18,19]. Low level exposure to 60% of all herbicides applied in the US are known to interfere with thyroid function, in particular 2,4-dichlorophenoxacetic acid (2,4-D) [20]. Glyphosate, another thyroid hormone disrupting herbicide [21], has also been shown to chelate multiple minerals essential to normal fetal development and health of adult animals, and to disrupt retinoic acid [22,23]. A large number of field studies have “found an association between exposure to environmental contaminants and alterations in thyroid gland structure, circulating thyroid hormones and vitamin A (retinoid) status” in multiple populations of wild vertebrates [23]. The proper quantity of minerals, retinoic acid and thyroid hormones are essential to normal development and growth as well as sustaining health during the life of the animal. Thus, exposure to environmental contaminants often results in “combreceptive and developmental dysfunction” in all vertebrate classes [24].

In this paper, we present some of the evidence Hoy has gathered, and we use it to inspire investigations on human health status in the general US population. While the animals’ exposure is likely mostly through water and air, we believe that human exposure is predominantly through food, as the majority of the population does not reside near agricultural fields. We have obtained government data on pesticide usage from the USDA and on human disease patterns over time from the CDC’s hospital discharge data, available from 1998 to 2010. Since glyphosate is by far the most widely used herbicide, we believe it to be a major source of contamination for the humans, and any correlations between glyphosate usage over time and specific health issues is likely to reflect a causal relationship. The research literature can help to clarify whether conditions whose incidence is rising in step with rising glyphosate usage could plausibly be caused by glyphosate, given its known toxicology profile.

Most of our graphs illustrating human disease patterns involve infants, but we also present evidence from children 0-15 and from the full population excepting newborns. We found many diseases and conditions whose hospital discharge rate over the twelve-year period match remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops. These include head and face anomalies (R=0.95) (Figure 10), newborn eye disorders (Figure 13), newborn blood disorders (Figure 15) (R=0.92), newborn skin disorders (R=0.96) and skin disorders in the general population (R=0.90). (Figure 17), lymph disorders in children 0-15 (R=0.86) and in the general population (R=0.89) (Figure 19), congenital heart conditions in newborns (R=0.98) and enlarged right ventricle in all age groups except newborn (R=0.96) (Figure 22), newborn lung problems (R=0.95) and pulmonary bleeding and edema for all age groups except newborn (R=0.97) (Figure 23), liver cancer (R=0.93) (Figure 25), newborn genitourinary disorders (R=0.96) (Figure 31) and newborn metabolic disorders (R=0.949) (Figure 32).

Glyphosate’s established mode of action in killing weeds is through disruption of the shikimate pathway [22,25] whose products, the essential aromatic amino acids, are important precursors to multiple biologically important molecules, including the neurotransmitters dopamine, serotonin, melatonin, and epinephrine, the B vitamin, folate, the molecule nicotineamine dinucleotide (NAD) involved in many redox reactions, and the tanning pigment, melanin [24,26]. Gut microbes produce the aromatic amino acids using the shikimate pathway, so this ability is impaired in the presence of glyphosate. A general mode of action of glyphosate is that it chelates the soluble ions of many mineral nutrients including calcium, copper, iron, magnesium, nickel and zinc, which are essential cofactors in many specific biochemical reactions [25,27]. Glyphosate has been shown to disrupt the gut microbiome in animals, probably in part through disrupting mineral bioavailability, including manganese, iron, zinc, and cobalt [22,24]. Impaired manganese homeostasis can explain many features of disorders whose incidence is rising in the human population, including autism, Alzheimer’s disease, Parkinson’s disease, osteoporosis, and rheumatoid arthritis [28]. Multiple pathogenic infections due to gut dysbiosis are a major factor in the decline in orcas (Orcinus orca) along the north Pacific coast of the US [29], and glyphosate exposure is a likely contributor.

The newborn is highly susceptible to oxidative stress produced by free radicals [30-32]. An excess of free radicals is implicated in neonatal chronic lung disease [33], which rose sharply in the newborn population in 2006 and was highly correlated with glyphosate usage (Figure 23). Inflammation, hypoxia, ischemia, glutamate, and free iron magnify the effect of free radicals [30]. Glyphosate suppresses the first step in the synthesis of the pyrrole ring, a core structural component of heme [34-36], leading to excess bioavailability of free iron. Glyphosate also, through its chelation of manganese, disrupts the synthesis of glutamate from glutamate, because the enzyme glutamine synthase depends on manganese as a catalyst [28]. Glyphosate can be expected to induce hypoxia by interfering with hemoglobin synthesis. Furthermore, melatonin is a highly effective antioxidant [32], but its...
Evidence of increased toxicity of glyphosate formulations

The toxicity experiments used by regulatory agents to decide whether to approve a new chemical explicitly require that the active ingredient be evaluated only in isolation [45]. Glyphosate formulations are trade secrets, but they often contain other ingredients that either make glyphosate itself more toxic to cells or are themselves innately toxic [46,47]. Polyethoxylated tallowamine (POEA) is used in many formulations as a common surfactant to improve glyphosate’s effectiveness. By 2006, nearly all of the glyphosate usage was in the form of the salt formulations. Other herbicides were also converted to salt formulations, including 2,4-D and Picloram. With continuously increasing use of the herbicide salt formulations, the symptoms of fetal hypothyroidism and multiple mineral deficiencies have increased alarmingly in wildlife.

Studies on rat liver mitochondria revealed that Roundup at 15 millimolar concentration collapsed the transmembrane potential, caused mitochondrial swelling and depressed respiration by 40% [48]. Glyphosate alone did not exhibit this effect. In vitro studies showed that only 1 to 3 ppm of POEA is enough to produce toxic effects on cellular respiration and membrane integrity [49]. The lipophilic character of POEA gives it the ability to penetrate cell membranes, and probably also enables glyphosate to gain access to cells. In addition, the salt-based formulations are suspected to be much more deadly to humans who attempt suicide through glyphosate ingestion [49].

Both glyphosate and chlorothalonil suppress cytochrome P450 (CYP) enzyme activity, resulting in a gradual depletion of the vital functions in the liver performed by the CYP enzymes [26,50]. CYP enzymes are responsible for the activation of Vitamin D, and they play a role in the production of bile acids and the synthesis and/or metabolism of cholesterol, testosterone, estrogen, progesterone and other corticosteroids. The suppression of CYP enzymes in the liver can be expected to greatly increase the toxicity of all xenobiotics to the liver, but it also has serious adverse effects on the immune system and other organ functions, including the reproductive organs [28]. While the fatality rate for glyphosate attempted suicide or accidental exposure had been relatively low in earlier reports, a paper published in 2008 claimed a fatality rate of nearly 30% [51]. Symptoms associated with human acute poisoning with glyphosate included respiratory distress, altered consciousness, pulmonary edema, shock, dysrhythmia, and renal dysfunction. Pulmonary and renal toxicity lead to mortality in humans, following metabolic acidosis and tachycardia [49]. Exposure of glyphosate to piglets in controlled experiments showed that the POEA-based formulation was much more toxic to the piglets [52]. Multiple adverse cardiovascular effects were observed, including pulmonary hypertension, circulatory collapse, and acute metabolic acidosis.

Additionally, in spring of 2006, a relatively new class of neonicotinoid insecticides, which bear a chemical resemblance to nicotine, began being used throughout the US and in other countries. These may well have synergistic effects with glyphosate, due to glyphosate’s suppression of CYP enzymes, which are needed for detoxification of neonicotinoids [53]. Our own observations on multiple disease trends in the US population reveal a sharp increase in hospital discharge rates for the health problems addressed herein around the 2006 time frame, which we hypothesize may be connected to the widespread switch to glyphosate salt-based formulations, as well as the introduction of neonicotinoids.

There was a corresponding increase after 2005 of birth defects and serious health problems on white-tailed deer fawns and other animals. This included a significant increase in enlarged right heart ventricle, lung damage, dilated lymphatic vessels on the heart surface and underdeveloped or damaged thymus on newborn white-tailed deer.
necropsied by Hoy. The original formulation of glyphosate had been shown to cause dilated heart on rabbit fetuses, and the percentage of rabbit fetuses with dilated heart was significantly elevated at all dose levels along with skeletal variations, anomalies and malformations [54]. We also observed congenital heart conditions in newborns as well as impaired lung function and enlarged right ventricle in human data (Figures 22 and 23), trending upward in step with glyphosate usage.

An extremely serious health issue with the hooves of wild ruminants began around 2007 in many areas of the United States and Canada. Moose, elk, deer, bighorn sheep and possibly other wild ungulates were observed to have disrupted growth of the keratin of the hooves, causing malformed hooves, severe lameness and resultant mortality. Laminitis has been increasing in horses throughout the United States. The keratin of the hooves of ungulates has a significant amount cholesterol sulfate in its composition, as shown in tests of horse hooves [55]. Impaired cholesterol sulfate synthesis appears to be a primary toxicity path of glyphosate [26].

Below, we will discuss some of our specific findings in more detail and link them to the research literature on animal exposures and on the effects of glyphosate and other pesticides on biological systems.

**Congenital head and facial malformations**

Glyphosate’s mineral chelating effects result in vital minerals being unavailable to the developing cells of vertebrate young. A primary mode of glyphosate action is chelating manganese. The most common birth defect on the white-tailed deer fawns is brachygnathia superior [10], shown to be caused by fetal mineral deficiencies, particularly manganese deficiency [56-58]. This is likely connected to the important role that manganese plays as a catalyst in the production of chondroitin sulfate, which is crucial for bone development [28,59].

Given the documented increase in incidence of underdeveloped facial bones, it appears that young of both bird and mammalian species are being affected by an agent, or more likely a combination of agents, that interfere with bone growth. Our studies on the CDC hospital discharge data revealed that human infants show a rise in disorders of mineral metabolism, specifically for the three minerals, calcium, magnesium, and phosphorus (Figure 32). Human infants are also experiencing an increase in anomalies of the head and face that matches well with glyphosate usage (Figure 10). Many environmental toxins have been shown to interfere with intracellular calcium levels and bone growth in developing animals [60]. Exposure of a mammalian fetus to pesticides more than doubles the risk of mortality due to developmental malformations [61]. Exposure of bird embryos to dioxin resulted in malformed skulls and brains [62]. It is likely that disruption of both calcium and energy metabolism would have an adverse influence on normal ossification, resulting in the underdevelopment of the skull, maxilla, leg bones and more rarely other skeletal bones, as has been observed on wildlife.

**Congenital thymus malformations, lymph system and thyroid**

The thymus of animals exposed to toxic pesticides is often very obviously damaged upon postmortem examination, as illustrated in Figure 14. Thymus and spleen development take place mainly in the postnatal period, and zinc deprivation during this critical time in mice can result in a markedly reduced size of the thymus [63,64]. Thymus involution due to apoptosis has also been implicated in association with magnesium deficiency [65,66]. Glyphosate’s chelating effects on +2 cations could lead to zinc and magnesium deficiency in exposed individuals. Glyphosate has been shown to deplete zinc as well as manganese in glyphosate-resistant soy crops [67]. Studies on rats have shown that melatonin, a product of the shikimate pathway, protects the thymus from oxidative damage [68].

Monsanto’s own studies showed that exposure of albino rats to a dust aerosol containing pure glyphosate for four hours led to lesion development on the lungs and thymus in the form of red foci [69]. These align well with the red spots that were observed on thymuses from newborn white-tailed deer in our Figure 14C, 14D and 14E.

Impairment in the thymus logically leads to disorders of the lymph system, which have increased dramatically, especially since 2006, in both children and the general population (except newborn) (Figure 19). Magnesium deficiency is linked to impaired immune function [70].

The thyroid modulates endocrine activity of the thymus, and thymulin levels are correlated with thyroxin 3 (T3) and T4 levels [71]. Human hypothyroidism may therefore be related to the observed defects in thymuses of animals exposed to toxic chemicals. The trend over time of hypothyroidism among children aged 0-15 aligns remarkably well with brachygnathia in deer fawns (Figure 11). Low magnesium was shown to decrease production of the most important form of Vitamin D, essential in bone development [72]. We hypothesize that these two patterns may be linked through manganese dysbiosis. In [28], it was proposed that glyphosate leads to an excess of magnesium in the brain stem and a deficiency in the vasculature, due to impaired bile flow in the liver. Excess magnesium in the brain stem has been hypothesized to damage thyroid function both through direct damage to the thyroid and through dysregulation of dopaminergic modulation of thyroid hormone synthesis [73].

Newborn rats in a multi-dose study showed developmental effects and delayed sexual maturation at all doses of Chlorothalonil [74]. Chlorothalonil is a nitrite as are its metabolites. It consists of two cyanide molecules attached to a hexachlorobenzene ring. Cyanide has been shown to disrupt thyroid hormone functions, especially during fetal development [75]. Additionally, many herbicides, particularly 2,4-D and Dicamba, disrupt normal thyroid hormone function [20], thus a cumulative or synergistic effect between the organochlorine pesticides and glyphosate should be considered.

**Reproductive system**

Endocrine disruption is trans-generational because a mother can accumulate toxic chemicals in fat tissues over many years, which are mobilized during pregnancy and lactation, to cause harm to the fetus or infant [76]. Each stage in the development of fetal reproductive organs requires precise amounts of hormones and enzymes, in addition to other factors, such as temperature, in some species of vertebrate. An alarming study of deep-water fish in the Bay of Biscay (northeast Atlantic Ocean) published in March 2015, found a wide variety of inflammatory and degenerative lesions in all species examined, in addition to diseases of the liver, and the first case of an intersex deep-water fish [76]. In a study of pollutants in National Parks, male fish were found to have female sex organs caused by pesticides in high mountain lakes in Glacier National Park, considered to be a pristine area, only150 miles north of our wildlife study area [77].

Conversion of testosterone to estrogen by aromatase depends on CYP enzymes. Aromatase activity is decreased by glyphosate [78]. Glyphosate also decreases serum testosterone concentrations.
Exposure to the commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology in prepubertal males [79]. Glyphosate also inhibits steroidogenesis and other normal functions of adult male reproductive organs, including the testicular cells [80,81].

Glyphosate caused cytotoxicity to progesterone-producing cells in vitro at levels that were comparable to the allowable levels in drinking water, leading to a decrease in progesterone production, and Roundup was more toxic than glyphosate [82]. Endocrine disrupting effects of Roundup on human female cells, and the activity of the pituitary-derived regulatory gonadotrophin, luteinising hormone (LH), and embryo-derived chorionic gonadotrophin (CG) activity, have not been sufficiently examined and may be contributing to the low reproductive rates in many wildlife species.

Glyphosate working synergistically with other pesticide exposures, disrupting normal hormone and enzyme levels and/or functions at key periods during fetal development, are the most likely cause of the variety of birth defects found in white-tailed deer and other wild ruminant populations since spring 1995 [10,11].

The decreased aromatase activity caused by glyphosate and possibly other pesticides may be responsible for the highly skewed sex ratio in favor of males found in Western Montana white-tailed deer fawns [10]. Studies considering the maternal condition prior to conception provide strong evidence for a relationship between maternal condition and the sex ratio in mammals [83], particularly in wild ungulates such as white-tailed deer [84]. Mineral deficiencies, damaged mitochondria and hormone disruption would certainly have an adverse effect on the condition of a pregnant female, especially in the wild. The sex ratio significantly skewed in favor of males began occurring in the Western Montana white-tailed deer fawns the same spring as the birth defects [85]. Most importantly, in 1995, marine mammals and vertebrates in other areas began being documented with unusual health problems and high rates of mortality in breeding age females and newborns [86,87].

**Congenital urogenital malformations**

Since 1995, an increasing prevalence of male reproductive malformations [16,24] has been observed on multiple vertebrate species. Analogous birth defects on vertebrates have more recently been shown to be the result of glyphosate exposure [88] as well as other pesticides [42]. Birth defects have been observed on multiple mammalian species [89], including human newborns [90], many individuals of multiple bird species [91,92], on reptiles, particularly alligators [93-95], and on multiple species of amphibian [96-98].

Disrupted development of the male genitalia, resulting in shortening of the penis sheath and/or the scrotum on the external skin, has been shown to be caused by a combination of zinc deficiency, disruption of retinoic acid (Vitamin A), Congenital Fetal Hypothyroidism (CFH) and dihydrotestosterone (DHT) disruption, all factors symptomatic of exposure to glyphosate [26,88].

Zinc deficiency in both a pregnant female and her male fetus or fetuses is likely a contributing cause of the shortening of the penis sheath, the underdevelopment of one or both hemiscrota, and possibly of the misalignment of the hemiscrota [99,100]. Cellular zinc levels have a strong influence on the 5-alpha reductase inhibitor, which converts testosterone into DHT. DHT is instrumental in the normal growth and development of external male reproductive organs because DHT bonds to androgen receptors more effectively than other natural androgens [101]. In addition, low levels of retinoic acid (vitamin A) have been connected to zinc deficiency in developing fetuses [102,103]. Also, retinoic acid receptor alpha, a receptor for retinoic acid, has profound effects on vertebrate development by directly regulating gene expression [104].

The disruption of vitamin A caused by ingesting glyphosate [88] would likely have serious effects in the digestive system of ruminants, which may be why they appear to be highly affected by health problems and birth defects. In ruminants, significant amounts of vitamin A are degraded in the rumen, while digestibility of carotene varies in different species [105]. There are also several variables that influence carotene digestibility and vitamin A content in forage including the type of forage, the plant species ingested and the month forage is eaten, being above average during warmer months and below average during the winter. Vitamin A levels depend upon adequacy of dietary fat, protein, zinc, phosphorus and antioxidants, which can be seriously lacking in the diets of wild ruminants in winter when the females are carrying developing fetuses. Vitamin A deficiency has been shown to cause lung and liver damage in rats [106].

With increasing use of glyphosate, the amount of glyphosate and other toxins in or on the ingested foliage is likely a primary factor affecting zinc and retinoic acid levels. Depending upon their size, ruminants ingest a large amount of foliage each day, resulting in consumption of biologically significant levels of glyphosate. Cellular zinc levels have a strong influence on the 5-alpha reductase inhibitor, which converts testosterone into DHT. DHT is instrumental in the normal growth and development of external male reproductive organs because DHT bonds to androgen receptors more effectively than other natural androgens [107].

Glyphosate and its synergistic effects with other pesticides, such as Chlorothalonil, are likely closely connected to the increasing prevalence of birth defects and health problems affecting the male reproductive organs since 1995 [10,11,85]. For example, genital hypoplasia, now very common, was almost unknown on white-tailed deer in years prior to 1995 [108] (Figures 28-30). A Danish study showed a steady increase in the incidence of hypospadias in boys from 1977 to 2005 [109]. Glyphosate became available on the market in 1975. Our own data show remarkable correspondence between newborn genitourinary defects, including hypospadias, and glyphosate usage on crops (Figure 31) (R=0.96).

Thyroid hormone disrupting chemicals act synergistically such that the combined effects are greater than linear. It can be expected that simultaneous fetal exposure to glyphosate and chlorothalonil would synergistically suppress CYP enzymes, as well as thyroid hormone functions, likely resulting in even more severe teratogenic effects than that caused by exposure to either alone. Added to the depletion of cellular zinc and the disruption of gonadotropin expression caused by glyphosate [78,110] the result is a significant assault on the growth and development of the male genitalia.

**Conclusion**

Something is causing alarming increases in diseases and birth defects in wildlife. Something is causing alarming increases in diseases and birth defects in humans. Our graphs illustrating human disease patterns over the twelve-year period correlate remarkably well with the rate of glyphosate usage on corn, soy, and wheat crops.

Glyphosate is known to chelate vital minerals [US Patent #3160632 A]. Glyphosate is an anti-bacterial agent [US Patent #20040077608 A1]. Glyphosate has been classified as an endocrine

disruptor by the Endocrine Society. Glyphosate has been classified as “probably carcinogenic” by the World Health Organization and by the American Cancer Society. Glyphosate interferes with the shikimate pathway, essential to healthy gut microbes. Glyphosate inhibits the CYP enzyme activity, which is vital to a healthy functioning liver.

The strong correlations between glyphosate usage and disease patterns, the highly significant p-values and the known toxicological profile of glyphosate indicate that glyphosate is likely a major factor in the increases in the serious issues with human health documented here.

Our over-reliance on chemicals in agriculture is causing irreparable harm to all beings on this planet, including the planet herself. Most of these chemicals are known to cause illness, and they have likely been causing illnesses for many years. But until recently, the herbicides have never been sprayed directly on food crops, and never in this massive quantity. We must find another way.

References

3. Swanson N, Leu A, Abrahamson J, Wallet B W, Graham JA (2006) 2. Monsanto B (2012) Preharvest Staging Guide. 1. Benbrook CB (2012) Impacts of genetically engineered crops on pesticide use causing illnesses for many years. But until recently, the herbicides have these chemicals are known to cause illness, and they have likely been harm to all beings on this planet, including the planet herself. Most of the increases in the serious issues with human health documented here.


