

## Glyphosate, pathways to modern diseases IV: cancer and related pathologies

Anthony Samsel<sup>1,\*</sup> and Stephanie Seneff<sup>2,\*\*</sup>

<sup>1</sup> Research Scientist, Deerfield, NH 03037, USA

<sup>2</sup> Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA 02139, USA

Glyphosate is the active ingredient in the pervasive herbicide, Roundup, and its usage, particularly in the United States, has increased dramatically in the last two decades, in step with the widespread adoption of Roundup®-Ready core crops. The World Health Organization recently labelled glyphosate as “probably carcinogenic.” In this paper, we review the research literature, with the goal of evaluating the carcinogenic potential of glyphosate. Glyphosate has a large number of tumorigenic effects on biological systems, including direct damage to DNA in sensitive cells, disruption of glycine homeostasis, succinate dehydrogenase inhibition, chelation of manganese, modification to more carcinogenic molecules such as N-nitrosoglyphosate and glyoxylate, disruption of fructose metabolism, etc. Epidemiological evidence supports strong temporal correlations between glyphosate usage on crops and a multitude of cancers that are reaching epidemic proportions, including breast cancer, pancreatic cancer, kidney cancer, thyroid cancer, liver cancer, bladder cancer and myeloid leukaemia. Here, we support these correlations through an examination of Monsanto’s early studies on glyphosate, and explain how the biological effects of glyphosate could induce each of these cancers. We believe that the available evidence warrants a reconsideration of the risk/benefit trade-off with respect to glyphosate usage to control weeds, and we advocate much stricter regulation of glyphosate.

**Keywords:** cataracts, CYP 450 enzymes, glyphosate, gut microbiome, interstitial disease, kidney cancer, non-Hodgkin’s lymphoma, pancreatic cancer

### 1. INTRODUCTION

Glyphosate is the active ingredient in the pervasive herbicide, Roundup. Its usage on crops to control weeds in the United States and elsewhere has increased dramatically in the past two decades, driven by the increase over the same time period in the use of genetically modified (GM)<sup>1</sup> crops, the widespread emergence of glyphosate-resistant weeds among the GM crops (necessitating ever-higher doses to achieve the same herbicidal effect), as well as the increased adoption of glyphosate as a desiccating agent just before harvest. GM crops include corn, soy, canola (rapeseed) and sugar beet [1]. Crop desiccation by glyphosate includes application to non-GM crops such as dried peas, beans and lentils. It should be noted that the use of glyphosate for pre-harvest staging for perennial weed control is now a major crop management strategy. The increase in glyphosate usage in the United States is extremely well correlated with the concurrent increase in the incidence and/or death rate of multiple diseases, including several cancers [1]. These include thyroid cancer, liver cancer, bladder cancer, pancreatic cancer, kidney cancer and myeloid leukaemia, as shown in Table 1, reproduced from [1]. The World

Health Organization (WHO) revised its assessment of glyphosate’s carcinogenic potential in March 2015, relabelling it as a “probable carcinogen” [2, 3].

Table 1. Pearson’s coefficients between time trends in various cancers and glyphosate applications to corn and soy crops, over the interval from 1990–2010, along with corresponding *P*-values, as determined from hospital discharge data and death data maintained by the US Centers for Disease Control (CDC). Table adapted from Swanson et al. 2014 [1].

Disease	<i>R</i>	<i>P</i>
Thyroid cancer (incidence)	0.988	$\leq 7.6 \times 10^{-9}$
Liver cancer (incidence)	0.960	$\leq 4.6 \times 10^{-8}$
Bladder cancer (deaths)	0.981	$\leq 4.7 \times 10^{-9}$
Pancreatic cancer (incidence)	0.918	$\leq 4.6 \times 10^{-7}$
Kidney cancer (incidence)	0.973	$\leq 2.0 \times 10^{-8}$
Myeloid leukaemia (deaths)	0.878	$\leq 1.5 \times 10^{-6}$

Sri Lanka’s newly elected president, Maithripala Sirisena, banned glyphosate imports as one of his first acts following election. This action was based on studies by Jayasumana et al. that provided compelling evidence that glyphosate was a key factor in the chronic kidney disease that was affecting an alarming number of young

\* E-mail: anthonyamsel@acoustictracks.net

\*\* Corresponding author. E-mail: seneff@csail.mit.edu

<sup>1</sup> Usually called genetically engineered (GE) in the USA.

agricultural workers in the northern region [4, 5], and was probably further motivated by the WHO reevaluation of its carcinogenic potential. Kidney disease is a risk factor for multiple cancers, with kidney dialysis being associated with increased risk of Kaposi's sarcoma by more than 50-fold, with 3- to 10-fold increased risk of kidney cancer, and 2- to 9-fold increased risk of thyroid cancer. Many other cancers also show more modest risk increases [6].

A study of rats fed GM maize and/or Roundup in their water over their entire lifespan revealed significantly increased risk of massive mammary tumours in the females, along with kidney and liver damage in the males [7]. Most of the tumours were benign, but there were three metastases (in female animals) and two Wilm's tumours found in the kidneys of males, which had to be euthanized early due to the excessive tumours, which grew to more than 25% of their body size. The exposed animals also had a shortened life span compared to the controls.

The hormone oestrogen was declared to be a human carcinogen by the National Toxicology Program in 2003 [8]. Glyphosate has been demonstrated to have oestrogenic effects at minute dosages, in *in vitro* experiments on mammary tumour cells [9]. Glyphosate was able to induce proliferation in these cells in concentrations of parts per trillion,<sup>2</sup> and it did so through binding affinity to the oestrogen receptor and inducing activation of the oestrogen response element (ERE). The fact that an oestrogen antagonist, ICI 182780, could inhibit glyphosate's action demonstrated rather conclusively that it was mediated through oestrogen mimicry.

Traditional concepts in toxicology are centred on Paracelsus' dictum that "the dose makes the poison", meaning that one should expect an increasing risk of toxicity as the level of exposure is increased. However, the generality of this concept has been challenged due to the realization that endocrine-disrupting chemicals (EDCs) often show a greater potential to cause cancer at very low doses than at higher doses; i.e., the relationship between dose and response is nonmonotonic, with higher doses producing a lower toxic effect than lower doses. In fact, levels of exposure well below the lowest level used in standard toxicology studies can be carcinogenic, as discussed by Vandenberg et al. [10]. These authors concluded their abstract as follows: "We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental

exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health."

Glyphosate is toxic to many microbes as well as to most plants, and one likely effect of chronic low-dose oral exposure to glyphosate is a disruption of the balance among gut microbes towards an over-representation of pathogens [11]. This leads to a chronic inflammatory state in the gut, as well as an impaired gut barrier and many other sequelae. It has become increasingly apparent that chronic inflammation increases cancer risk and, in fact, many inflammatory conditions, such as Crohn's disease, hepatitis, schistosomiasis, thyroiditis, prostatitis and inflammatory bowel disease are known cancer risk factors [12].

In this paper, we review the research literature on glyphosate, with particular emphasis on evidence of carcinogenic potential, which includes glyphosate's induction of metabolic disorders, oxidative stress and DNA damage, known precursors to cancer development. We begin with a section that summarizes our own findings following perusal of large numbers of documents that were provided to one of us (Samsel) by the US Environmental Protection Agency (EPA), according to the Freedom of Information Act, which provided detailed information on Monsanto's own early experimental animal studies on glyphosate.

This section motivates and inspires subsequent sections where we seek to explain the likely mechanisms by which glyphosate might cause the tumours observed in Monsanto's studies as well as explaining the strong statistical correlations with human cancers. Following a section that provides direct evidence of DNA damage, the next four sections discuss metabolic disorders linked to glyphosate that are known to increase cancer risk, including succinate dehydrogenase inhibition, glycation damage, N-nitrosylation, and disrupted glycine homeostasis. The subsequent eight sections successively address cancer of the colon, liver, pancreas and kidney, melanoma, thyroid cancer, breast cancer, and lymphoma. In each section we provide evidence of a link to glyphosate from the research literature and propose plausible explanations for a causal link. We finally conclude with a summary of our findings.

<sup>2</sup> U.S. trillion, i.e.  $10^{12}$ .

#### 4. MONSANTO'S EARLY STUDIES

One of us (Samsel) petitioned the EPA for copies of documents originating from Monsanto, dating from the 1970s through the 1980s, which described experiments conducted by Monsanto to evaluate whether glyphosate is safe for human consumption. In this section, we provide a summary of our findings related to those documents, especially with respect to indications of kidney damage, tumorigenicity, bioaccumulation, and glyphosate metabolites.

##### 4.1 Kidney damage

Classification of types of kidney damage, which are indicative of kidney disease, are noted below, based on information contained in Monsanto's glyphosate studies on rats and mice [13–18]. In [13], changes in the kidneys associated with chronic progressive neuropathy were noted mostly in males, but also in some female animals of both control and treated groups. There was also mineralization and mineralized debris found in the pelvic epithelium of the kidney, most often in females.

Following submission of the study, the EPA subsequently asked Monsanto for a histological re-examination of the low- and mid-dose male animals, which resulted in establishing a no observable effect level (NOEL). In response, Monsanto submitted an addendum [14] to the pathology report. The results of the addendum summarized the examination of the kidneys and found minimal tubular dilatation accompanied by interstitial fibrosis in all test groups. Statistically significant increases in tubular dilatation of the kidney were noted. A 50% increase in changes to the kidney of the low-dose group and, in the high-dose group, a fourfold increase in incidence was found compared to the control.

Interstitial renal fibrosis begins with an accumulation of extracellular matrix proteins, which is the result of inflammation and injury to the cells, which is found in every type of chronic kidney disease (CKD). Interstitial fibrosis is a progressive pathogenesis leading to end-stage renal failure [19].

The results of the 1981 study [17] further found:

1. Focal tubular hyperplasia, a hyperplasia of the tubular epithelium of the kidney caused by repeated tubular damage. It is characterized by an abnormal increase in the number of cells, which causes enlargement. Tubular epithelial hyperplasia precedes the pathogenesis of tubular dilatation in acute tubular necrosis [20].
2. Focal tubular dilation, a swelling or flattening of the renal tubule, seen as a result of an ischaemic or toxic event as in pharmaceutical, antibiotic or chemical poisoning. This leads to acute tubular necrosis, a cause of acute kidney injury and kidney failure.

3. Focal tubular nephrosis, a degenerative disease of the renal tubules of the kidney. This nephrosis is a noninflammatory nephropathy that features damage of the renal tubules [21].

4. Interstitial mononuclear cell infiltrate characteristic of inflammatory lesions, which consist of white blood cells that clear debris from an injury site.

Mineral deposits can be indicative of kidney stones, which may be calcium oxalate deposits inside the kidney, as we shall discuss more fully later in this paper.

A 1983 chronic feeding study in mice [16] found a carcinogenic response to glyphosate in both male and female mice. There was also an increased incidence of chronic interstitial nephritis in male animals. The study, lasting 18 months, involved feeding glyphosate by diet using concentrations of 1000, 5000 and 30 000 ppm. The incidence of kidney tumours in the control animals was 0/49, as was also noted in the lowest dose group. However, the mid-dose and high-dose groups produced incidences of neoplasms at 1/50 and 3/50 respectively, which caused the EPA Oncogenicity Peer Review Committee to temporarily classify glyphosate as a Class C carcinogen.

Monsanto, dissatisfied with the action, consulted another pathologist who, upon further examination, found a small tumour in the control. This was followed by the EPA using a number of pathologists to re-examine additional kidney sections from the mice to check the validity of the findings. However, their re-examination did not find any additional tumours nor confirm the tumour in the control animal. There were no tumours present in any additional sections. EPA asked for the decision to be externally refereed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel, who found the results were statistically significant even after comparing the data to historical controls. However, the committee agreed to downgrade glyphosate to a Class D compound, arguing inadequate evidence of oncogenicity, and further sealed the study as a trade secret of Monsanto.

Non-neoplastic changes included:

1. Renal tubular neoplasms (in male mice; none found in females);
2. Chronic interstitial nephritis (in males);
3. Renal tubular epithelial basophilia and hyperplasias (decreased in males, but a dose-related increase found in females);
4. Proximal tubule epithelial cell basophilia and hypertrophy (females).

##### 4.2 Tumorigenicity

A 26-month long-term study in rats conducted by Bio/dynamics revealed multitudes of tumours in glands and organs [13]. They occurred (from highest to lowest

incidence) in the following organs: pituitary, thyroid, thymus, **mammary glands**, **testes**, kidney, pancreas, liver and lungs. Pituitary, thyroid and thymus glands control body and immune function, and disruption can induce disease, including cancer. These glands produce many necessary hormones that control numerous biological processes. Tumorigenic growth also disrupts functionality of the glands and organs where the growth occurs. A Monsanto trade secret document [13] revealed that there were statistically significant lymphocytic hyperplasias of the thymus as well as **significant** C-cell thyroid tumours. Thymus lymphoid hyperplasia occurs in Graves disease and thymus hyperplasia is commonly observed with computed tomography (CT) scans of thyroid cancer patients [22], and is also associated with autoimmune disorders such as myasthenia gravis, lupus erythematosus, scleroderma and rheumatoid arthritis [23].

It should be noted that significant incidence of tumours was found during these investigations. However, to create doubt and obscure the statistical significance of inconvenient findings, which may have prevented product registration, Monsanto used experimental noise from 3, 5, 7 and even 11 unrelated study controls to effectively eliminate results, as needed. In some instances the experiments' own control showed 0% incidence of tumours, while the results for the glyphosate-treated groups were statistically significant. However, through the dishonest magic of comparing the findings to data from unrelated historical controls, they were explained away as a mystery and deemed not to be related to administration of the glyphosate.

Using these deviations effectively neutralized the inconvenient results and thus allowed the product to be brought to market. Had they not engaged in this deception, glyphosate may never have been registered for use. EPA documents show that unanimity of opinion for product registration was not reached. Not all members of the EPA glyphosate review committee approved the registration of glyphosate. There were those who dissented and signed "DO NOT CONCUR."<sup>3</sup>

The EC GLP document [24] notes: "Misdosing and/or cross-contamination of the test item is always a risk in animal studies. These problems are usually detected by the presence of the test item and /or its metabolites in plasma or other biological samples from control animals. It is recognized that dietary and topical studies might lead to a higher level and incidence of contamination of test item in control animals. However, contamination of biological samples from control animals has been observed also in studies using other routes of administration, e.g. gavage, intravenous, intraperitoneal, subcutaneous or inhalation. Exposure of the control animals to the test item may compromise or invalidate the study from a scientific point of view."

Thus, these unrelated historical controls were most likely corrupted studies, whether by technician error, contaminated water and /or feed, or other mistakes. This explains Monsanto's collusion with the EPA and the subsequent hiding of the data from purview.

Data tables are presented in Tables 2 through 7, without the use of experimental noise from historical controls. Only the data results of the experiment are shown.

Table 2. 1981 Bio/dynamics 26-month glyphosate feeding study [17]: interstitial cell tumours of the testes in Sprague Dawley rats.

Glyphosate dose /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
Terminal sacrifice	0/15 (0%)	2/26 (7.69%)	1/16 (6.25%)	4/26 (15.38%)
All animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)

Table 3. 1981 Bio/dynamics 26-month glyphosate feeding study [17]. Incidence of kidney focal tubular dilatation (FTD) and focal tubular nephrosis (FTN) in Sprague Dawley rats.

Glyphosate dose /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
FTD unilateral	2/10 (20%)	3/10 (30%)	2/9 (22%)	7/10 (70%)
FTD bilateral	0/10 (0%)	2/10 (20%)	1/9 (11%)	1/10 (10%)
FTN unilateral	1/10 (10%)	2/10 (20%)	1/9 (11%)	0/10 (0%)
FTN bilateral	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)

<sup>3</sup> The practice of introducing "experimental noise" by using data from unrelated historical controls is still in use today, but is obviously really bad laboratory practice. The European Union Good Laboratory Practice (GLP) Working Group approved a guidance document for GLP inspectors and test facilities in 2005; it is available at the European Commission (EC) GLP internet site [24]. The document discusses the responsibilities of the study director and the principles of identifying misdosing as well as corrective measures.



Table 4. 1981 Bio/dynamics 26-month glyphosate feeding study [17]: incidence of pancreatic islet cell tumours in male Sprague Dawley rats.

Glyphosate dose /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
Adenomas	0/50 (0%)	5/49 (10%)	2/50 (4%)	2/50 (4%)
Carcinomas	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
Adenomas and carcinomas	0/50 (0%)	5/49 (10%)	2/50 (4%)	3/50 (6%)
Hyperplasias	3/50 (6%)	2/49 (4%)	1/50 (2%)	0/50 (0%)

Table 5. 1990 Stout & Rueker 24 month glyphosate feeding study [15]: incidence of pancreatic islet cell tumours in male Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	1/43 (2%)	8/45 (18%)	5/49 (10%)	7/48 (15%)
<i>P</i>	0.170	0.018	0.135	0.042
Carcinomas	1/43 (2%)	0/45 (0%)	0/49 (0%)	0/48 (0%)
<i>P</i>	0.159	0.409	0.467	0.472
Adenomas and carcinomas	2/43 (5%)	8/45 (18%)	5/49 (10%)	7/48 (15%)
<i>P</i>	0.241	0.052	0.275	0.108
Hyperplasia	2/43 (5%)	0/45 (0%)	3/49 (6%)	2/48 (4%)
<i>P</i>	0.323	0.236	0.526	0.649

Table 6. 1990 Stout & Rueker 24 month glyphosate feeding study [15]: incidence of thyroid C-cell tumours in male Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
<i>P</i>	0.069	0.348	0.060	0.099
Carcinomas	0/54 (0%)	2/55 (4%)	0/58 (0%)	1/58 (2%)
<i>P</i>	0.452	0.252	1.000	0.518
Adenomas and carcinomas	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
<i>P</i>	0.077	0.141	0.060	0.060
Hyperplasia	4/54 (7%)	1/55 (2%)	5/58 (9%)	4/58 (7%)
<i>P</i>	0.312	0.176	0.546	0.601

Table 7. 1990 Stout and Rueker 24 month glyphosate feeding study [15]: incidence of thyroid C-cell tumours in female Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	2/57 (4%)	2/60 (3%)	6/59 (10%)	6/55 (11%)
<i>P</i>	0.031	0.671	0.147	0.124
Carcinomas	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)
<i>P</i>	0.445	1.000	0.509	1.000
Adenomas and carcinomas	2/57 (4%)	2/60 (3%)	7/59 (12%)	6/55 (11%)
<i>P</i>	0.033	0.671	0.090	0.124
Hyperplasia	10/57 (18%)	5/60 (8%)	7/59 (12%)	4/55 (7%)
<i>P</i>	0.113	0.112	0.274	0.086

In a long-term study conducted by Monsanto between 1987 and 1989 [15], glyphosate was found to induce a statistically significant ( $P < 0.05$ ) cataractous lens formation, highest in male rats. Considerably higher doses of glyphosate, i.e., 2000, 8000 and 20 000 ppm, were administered to

low-, mid- and high-dose animals respectively, as compared to long-term studies conducted on mice and rats in the early 1980s. Over the course of the study, cataract lens changes were seen in low-, mid- and high-dosed groups of both male and female rats. A second pathology

examination also found statistically significant changes (see Table 8). The pathologist concluded that there was a glyphosate-treatment related response for lens changes

to the eyes. Monsanto documents also revealed an increased incidence of basophilic degeneration of the posterior subcapsular lens (fibroses) in highly dosed males.

Table 8. Incidence and occurrence of ophthalmic degenerative lens changes by glyphosate [15].

	Control	Low-dose	Mid-dose	High-dose
Male rats	2/14 (14%)	3/19 (16%)	3/17 (18%)	5/17 (29%)
All Animals	4/60 (7%)	6/60 (10%)	5/60 (8%)	8/60 (13%)

At the conclusion and termination of the experiment, further incidence of degenerative lens changes was revealed, as shown in Table 8. The ophthalmic examination yielded no noticeable changes to the control animals (0/15 or 0.0%); however, highly dosed males were significantly

impacted as 5/20 (25%), as shown in Table 9. The study again noted that “the occurrence of degenerative lens changes in high dose male rats appears to have been exacerbated by (glyphosate) treatment” [15]. Unrelated historical controls were used to negate all findings.

Table 9. Data on unilateral and bilateral cataracts (all types) and Y-suture opacities, excluding “prominent Y suture”, following glyphosate exposure to rats. From Stout & Rueker (1990) [15].

Sex	Group	No. Examined	No. Affected	% Affected
Male	N	15	0	0
	1	22	1	5
	2	18	3	17
	3	20	5	25
Female	N	23	0	0
	1	24	0	0
	2	17	1	6
	3	19	2	11

Stout & Ruecker [15] noted in a two year study with chronic feeding of glyphosate in rats: “Histopathological examination revealed an increase in the number of mid-dose females displaying inflammation of the stomach squamous

mucosa. This was the only statistically significant occurrence of non-neoplastic lesions.” Incidence of lesions of the squamous mucosa are shown in Table 10. Again, Monsanto used unrelated historical controls to negate these findings.

Table 10. Lesions of the stomach squamous mucosa in rats chronically exposed to glyphosate at three different levels (adapted from Stout and Ruecker [15].

	Controls	Low	Mid	High
Glyphosate (ppm)	0	2000	8000	20 000
Males	2/58 (3.44%)	3/58 (5.17%)	5/59 (8.47%)	7/59 (11.86%)
Females	0/58 (0.00%)	3/60 (5.00%)	9/60 (15.00%)	6/59 (10.16%)

### 4.3 Bioaccumulation

Ridley and Mirly [25] found bioaccumulation of <sup>14</sup>C-radiolabelled glyphosate in Sprague Dawley rat tissues. Residues were present in bone, marrow, blood and glands including the thyroid, testes and ovaries, as well as major organs, including the heart, liver, lungs, kidneys, spleen and stomach. Further details are shown in Table 11. A low-dose, oral absorption (10 mg/kg body weight) of the radiolabelled xenohormone indicated highest bioaccumula-

tions. The 1988 Monsanto study disclosed: “A significantly greater percentage of the dose remained in the organs and tissues and residual carcasses for the males than for the females. Overall recoveries for group 5 animals were 92.8% and 94.2% for males and females respectively.”

The study examined seven test groups of 3 to 5 animals per sex/group that were administered a single radiolabelled dose of glyphosate. Blood, expired air, faeces and urine were collected and analysed by liquid scintillation counting

(LSC), and glyphosate with its metabolites analysed by two methods of high pressure liquid chromatography (HPLC). Three animals were used in groups sacrificed at the end of 24 hours, and 5 animals were used for all other groups, which were sacrificed at the end of the seven day study. Groups 3 and 7 received a 10 mg/kg intravenous dose, while group 4 a high oral dose (1 g/kg). Groups 1, 2, 5 and 6 each received a single oral 10 mg/kg radiolabelled dose. Group 6 animals received multiple low doses of 10 mg/kg of nonradiolabelled glyphosate for 14 days prior to administration of a single 10 mg/kg radiolabelled dose.

Oral absorption of glyphosate was 30% and 35%,

with a radiological  $\beta$  half-life of 7.5 and 14 days in male and female animals, respectively. Bioaccumulation of glyphosate found in bone was 0.748 ppm for males and 0.462 ppm for females for group 6 animals. Group 5 animals retained 0.552 ppm and 0.313 ppm for males and females, respectively. Males also had higher levels of glyphosate in their blood. Approximately 0.27% of the orally administered dose was found in expired CO<sub>2</sub> of the group 1 rats sacrificed after 24 hours. Table 11 shows the mean average and percentage distribution of radioactivity in ppm that were found in tissues and organs of groups 4, 5 and 6 of the orally dosed animals.

Table 11. Distribution and bioaccumulation of <sup>14</sup>C radiolabelled glyphosate in blood, bone, glands, organs and other tissue of Sprague Dawley rats. Data obtained from Ridley & Mirly, 1988 [25] (see text for details).

Glyphosate mean (ppm)	Group 4		Group 5		Group 6	
	Male	Female	Male	Female	Male	Female
BLOOD						
Blood plasma	0.129	0.127	0.00158	0.00114	0.00178	0.00152
Red blood cells	0.517	0.275	0.00845	0.00424	0.00763	0.00474
Whole blood	0.328	0.166	0.00454	0.00269	0.00476	0.00288
Bone	30.6	19.7	0.552	0.313	0.748	0.462
Bone marrow	4.10	12.50	0.0290	0.00639	0.0245	0.0231
GLANDS						
Thyroid	1.50	1.24	0.000795	0.000358	0.00703	0.00955
Testes/ovaries	0.363	0.572	0.00276	0.00326	0.00529	0.00813
ORGANS						
Brain	0.750	0.566	0.00705	0.00551	0.0144	0.0110
Eye	0.655	0.590	0.00215	0.000298	0.00405	0.00337
Heart	0.590	0.518	0.00622	0.00398	0.00804	0.00632
Kidney	1.94	1.35	0.0216	0.0132	0.0327	0.0196
Liver	1.91	1.37	0.0298	0.0135	0.0407	0.0257
Lung	1.54	1.13	0.0148	0.0120	0.0211	0.0167
Spleen	2.61	2.98	0.0119	0.00727	0.0155	0.0130
Uterus	-	0.618	-	0.00517	-	0.00185
DIGESTIVE SYSTEM						
Stomach	2.38	2.36	0.00795	0.00367	0.0377	0.0239
Small intestine	1.90	1.55	0.216	0.0183	0.0441	0.0257
Colon	11.0	9.20	0.0342	0.0159	0.0429	0.0298
FAT/MUSCLE						
Abdominal fat	0.418	0.457	0.00364	0.00324	0.00557	0.00576
Testicular/ovarian fat	0.442	0.037	0.00495	0.00347	0.00721	0.00563
Abdominal muscle	0.262	0.214	0.00232	0.00160	0.00278	0.00216
Shoulder muscle	0.419	0.423	0.00388	0.00667	0.00783	0.00590
Nasal mucosa	1.71	1.79	0.00485	0.0226	0.0316	0.0125
Residual carcass	8.78	7.74	0.106	0.0870	0.157	0.101

#### 4.4 Glyphosate metabolites

Howe, Chott & McClanahan [26] identified, characterized and quantified glyphosate and its metabolites after intravenous and oral administration of the radiolabelled compound. They employed several analytical tools, including LSC, strong anion exchange (SAX), cation exchange (CX) and ion pair chromatography (IPC). CX and IPC methods of HPLC were used primarily for the identification of glyphosate and its metabolites contained in urine and faeces. Metabolites of glyphosate found during analysis include the nonbasic compounds aminomethylphosphonic

acid (AMPA), methylaminomethylphosphonic acid (MAMPA), N-formylglyphosate, N-acetylglyphosate, N-nitrosoglyphosate and an unknown compound tagged as "Compound #11". Metabolites found in the dosing solutions administered to rats of these experiments would be expected in all glyphosate-based products. CX analysis was used to identify AMPA and MAMPA and IPC was used to identify all other nonbasic glyphosate metabolites. Results are presented in Table 12. Metabolites were also found in the urine and faeces of both male and female rats, as shown in Table 13 for orally dosed groups 4, 5 and 6.

Table 12. Glyphosate and its metabolites: Analysis of dose solutions expressed as % of total. Table adapted from Howe et al. [26].

Dose group	Glyphosate	AMPA	MAMPA	N-acetyl-glyphosate	N-formyl-glyphosate	N-nitroso-glyphosate	Compound #11
1: Oral 10 mg/kg	98.21	0.63	0.26	<0.04	0.49	<0.05	<0.06
3: Intravenous 10 mg/kg	99.14	0.36	0.00	<0.02	0.36	<0.01	0.03
4: Oral 1000 mg/kg	98.88	0.57	0.31	<0.03	0.14	<0.02	0.04
5: Oral 10 mg/kg	99.41	0.17	0.00	<0.03	0.18	<0.03	0.03
6: Preconditioned Oral 10 mg/kg	99.36	0.19	0.07	<0.03	0.21	<0.02	<0.02

Table 13. Glyphosate and its metabolites: Analysis of faeces and urine from male and female rats expressed as % of total. Table adapted from Howe et al. [26].

Dose group	Glyphosate	AMPA (A)	MAMPA (M)	N-Acetyl-glyphosate	N-Formyl-glyphosate	N-Nitroso-glyphosate	Compound #11
4							
Dose solution	98.88	0.57	0.31	<0.03	0.14	<0.02	0.04
Male urine	97.76	1.25 A+M		0.10	0.20	0.09	0.46
Male faeces	98.64	0.82 A+M		<0.03	<0.04	0.13	0.16
Female urine	97.71	1.39 A+M		<0.05	0.25	0.09	0.33
Female faeces	98.68	0.88 A+M		<0.04	<0.04	0.11	0.17
5							
Dose solution	99.41	0.17	0.00	<0.03	0.18	<0.03	0.03
Male urine	99.05	0.32 A+M		<0.05	0.12	0.11	0.31
Male faeces	98.78	0.56 A+M		<0.06	<0.10	0.21	0.16
Female urine	98.65	0.30 A+M		<0.06	0.25	0.11	0.58
Female faeces	98.23	0.64 A+M		<0.05	<0.09	0.22	0.16
6							
Dose solution	99.36	0.19	0.07	<0.03	0.21	<0.02	<0.02
Male urine	99.24	0.29 A+M		<0.05	<0.11	0.08	0.18
Male faeces	98.31	0.90 A+M		<0.06	<0.10	0.24	0.17
Female urine	98.84	0.26 A+M		<0.04	0.12	0.15	0.51
Female faeces	98.27	0.93 A+M		<0.05	<0.10	0.22	0.23

*In vivo* metabolization of glyphosate to AMPA was found in the excreta in quantities  $\leq 0.4\%$ . The bone was the site of highest bioaccumulation and it retained 0.02 to 0.05% of the oral dose and 1% of the intravenous dose. Repetitive dosing of group 6 animals did not significantly change the metabolization or excretion of glyphosate. Of

all of the nonbasic compounds found during analysis of excreta, AMPA followed by N-nitrosoglyphosate were most prevalent. Total N-nitrosoglyphosate levels found in the animals ranged between 0.06–0.20% of the given dose. Faecal samples contained 0.10–0.32% and urine 0.06–0.15% of N-nitrosoglyphosate. Stability studies



revealed that the majority of the N-nitrosoglyphosate found in the faeces was not completely due to presence of the compound as a contaminant of glyphosate, nor was it due to animal metabolism, but rather was due to the chemical reaction of glyphosate with nitrites contained in the excreta. Glyphosate readily reacts with oxides of nitrogen (e.g., NO<sub>2</sub>) to form the metabolite N-nitrosoglyphosate. This engenders concern because N-nitroso compounds are carcinogens. Nitrous acid occurring in sweat excreta of the skin could be problematic in the presence of glyphosate and may be responsible for the rise of some skin cancers. N-nitrosoglyphosate, the product of chemical reaction between glyphosate residues and nitrites in the colon, may in fact be a causal agent in the alarming increase in colorectal cancer. We discuss N-nitrosoglyphosate in §8.

Colvin, Moran & Miller [27] evaluated the metabolism of <sup>14</sup>C-AMPA in male Wistar rats. A 6.7 mg/kg dose of radiolabelled AMPA was administered orally, of which 20% was found unchanged in the urine of the animals and 74% in the faeces. Recovery from excreta totalled 94% of the dose. In another study, Sutherland [28] fed Sprague Dawley rats a single radiolabelled dose of N-nitrosoglyphosate and successfully quantified the metabolite in the urine and faeces. Male and female animals received 3.6 mg/kg and 4.7 mg/kg, excreting 2.8% (faeces) 88.7% (urine) and 10.7% (faeces), 80.8% (urine) respectively. Both male and female rats retained 8.5% of the N-nitrosoglyphosate dose, while 90.5% was eliminated in excreta.

## 5. THE ISSUE OF CONTROL RATS' DIET

“Historical control data” show that 13–71% of the lab animals used to conduct toxicity tests on various chemicals would spontaneously present with mammary tumours, and 26–93% develop pituitary tumours. Their kidney function is also frequently impaired. A recent study by Mesnage et al. [29] sought to evaluate whether toxic chemicals present in the feed that is standard fare for these animals might be causative for this surprisingly high background rate of disease. Nine out of 13 samples of commonly used laboratory rat feeds tested positive for glyphosate. Thus, these “spontaneous” disease manifestations may well be due to the toxic chemicals in the feed in the control animals rather than to some underlying genetic defects, and this fact raises serious questions about the validity of any studies based on such exposed animals as a control group.

A 1995 paper by Dixon et al. describes a thorough analysis of the frequencies of various organ pathologies related to cancer and other diseases in “control” animals not subjected to any explicit administration of the toxic chemical under investigation [30]. The paper gave no information on the rats' feed or supplements, which would have been important as a possible confounding

factor in the observed pathologies, one of which was acinar cell atrophy, present in the pancreas of 6.9% of the males and 5.0% of the female rats. The authors noted a decrease in size and number of acini and increased amounts of interstitial tissue, suggesting fibrosis, along with increased infiltration of lymphocytes and macrophages. Since this is quite similar to the pathology observed with glyphosate exposure to rats, a possibility is that glyphosate contamination in their feed or water supply contributed to the pathology, perhaps in part by chelating manganese; this transition metal is known to stimulate protein synthesis in acini isolated from both diabetic and normal rats and, in the case of diabetic rats, the effect was shown to be specific to manganese (cobalt, nickel, barium, strontium and magnesium failed to exert the effect) [31].

To test for the hypothesis of glyphosate contamination in rat feed, we used HPLC to test for glyphosate and AMPA levels in three distinct rat chow products, containing corn, soy and wheat middlings, and found significant levels of both chemicals in all products examined. We also tested for choline and folic acid. As shown in Table 14, our laboratory analysis of standard rodent diets found no detectable folic acid. Folic acid (folate) is supplied not only through diet but also, particularly, by commensal bacteria via the shikimate pathway [32]. Therefore, glyphosate evidently disrupts folic acid production both in exposed plant food sources and in the human gut, leading to deficiencies. Folate is a cofactor in many important biologic processes, including remethylation of methionine and single carbon unit donors during DNA biosynthesis. This impacts gene regulation, transcription and genomic repair. Folate deficiency enhances colorectal carcinogenesis, in part through impaired DNA methylation [33]. Folate deficiency has also been implicated in the development of several cancers, including cancer of the colorectum, breast, ovary, pancreas, brain, lung and cervix [34]. Folate deficiency during gestation is linked to neural tube defects such as anencephaly and spina bifida.

A synthetic form of choline, choline chloride, has been added to formulated lab chow diets for decades, as indicated from historical references available from manufacturers such as Purina. A 2010 European patent application describes the addition of choline chloride to glyphosate formulations to act as a bioactivator and to enhance penetration of glyphosate into the cells of the target weed [35]. A study of 47,896 male health professionals in the US found that high choline intake was associated with an increased risk of lethal prostate cancer [36]. Our samples all tested positive for choline (see Table 14).

Table 14. Evidence of glyphosate contamination, and levels of folate and choline, in Purina rat chow products as determined from authors' own analyses.

	Glyphosate /mg kg <sup>-1</sup>	AMPA /mg kg <sup>-1</sup>	Folate /mg g <sup>-1</sup>	Choline /mg g <sup>-1</sup>
Purina Rat Chow 5002	0.65	0.35	0	4.827
Purina Chow 5K75	0.57	0.27	0	5.328
Purina Chow 5LG3	0.37	0.10	0	5.919

The American Veterinary Medical Foundation notes that "Cancer is the leading cause of death in older pets, accounting for almost half of the deaths of pets over 10 years of age." According to the Morris Animal Foundation, established in 1948, one in four dogs will die of cancer and over 22 000 cats will be diagnosed with aggressive sarcomas. Oral cancer squamous cell carcinomas are

now found in cats and lead to the destruction of the jawbone. Mammary tumours, a common cancer found in dogs and cats, are also on the rise. We suspect that glyphosate may be a causal agent related to the rise of pet cancers, and used HPLC to analyse 9 popular brands of dog and cat food. We found significant levels of both glyphosate and AMPA in all pet foods tested (Table 15).

Table 15. Glyphosate and AMPA residues found in various dog food and cat food products, as measured from samples tested by the authors.

	Glyphosate /mg kg <sup>-1</sup>	AMPA /mg kg <sup>-1</sup>
Purina Cat Chow Complete	0.102	0.12
Purina Dog Chow Complete	0.098	0.076
Kibbles-n-Bits Chefs Choice Am Grill	0.30	0.24
Friskies Indoor Delights	0.079	0.11
9 Lives Indoor Complete	0.14	0.12
Rachael Ray Zero Grain	0.022	Trace (< 0.02)
Iams Proactive Health	0.065	Trace (< 0.02)
Rachael Ray Nutrish Super Premium	0.14	0.14
Purina Beyond Natural - Simply Nine	0.047	0.031

Clearly, it is imperative that future studies on the potential toxicity of any environmental chemical address the issue of the possible toxicity of chemicals contaminating the diet of the control animals, and/or the potential impact of nutritional imbalances. Feeding the control animals an unhealthy diet leads to an increased risk of cancer in the control group making it much harder to see a signal in the experimental group. Furthermore, since oestrogenic chemicals are often more toxic at extremely low doses than at mid-range doses, it is easy to see why the control group may manifest a significant incidence of cancer.

## 6. EVIDENCE OF DNA DAMAGE FROM THE RESEARCH LITERATURE

According to the IARC's report [2], while there exists only limited direct evidence of carcinogenicity of glyphosate in humans, strong evidence exists to show that glyphosate can operate through two key features of carcinogens: induction of chromosomal damage and induction of oxidative stress. In this section, we review the evidence that glyphosate can damage DNA, a crucial

first step leading to cancer. We examine evidence based on sea urchins, children in Malaysia, in mouse models, both *in vitro* and *in vivo*, in human lymphocytes, and in fish. We conclude with a paragraph on folate deficiency, its probable link to glyphosate exposure, and folate's essential rôle in DNA maintenance.

Cell cycle disruption is a hallmark of tumour cells and human cancers. A study on sea urchins investigated several different glyphosate-based pesticide formulations, and found that all of them disrupted the cell cycle. The sprays used to disseminate pesticides can expose people in the vicinity to 500 to 4000 times higher doses than those needed to induce cell cycle disruption [37].

A study on children living near rice paddy farms in Malaysia revealed DNA strand breaks and chromosome breakage associated with reduced blood cholinesterase levels [38], which were attributed to exposure to organophosphate insecticides. The study did not specify exactly to which pesticides the children were exposed, but glyphosate is a general-purpose herbicide whose use in rice paddies in Sri Lanka led to widespread kidney

failure among young agricultural workers there, ultimately resulting in a ban on glyphosate usage in Sri Lanka [4, 5]. While glyphosate is technically an organophosphonate rather than an organophosphate, a study on the fish *Prochilodus lineatus* has demonstrated that it suppresses cholinesterase in both muscle and brain [39].

Bolognesi et al. [40] studied the genotoxic potential of both glyphosate in isolation and Roundup, in both mouse *in vivo* studies and *in vitro* studies of human lymphocytes. In the mouse studies they found evidence of DNA strand breaks and alkali-labile sites in liver but especially in kidney, as well as in bone marrow. Roundup was found to be more toxic than glyphosate, with damage occurring at lower concentrations. They also demonstrated dose-dependent sister chromatid exchanges in human lymphocytes exposed to glyphosate and to Roundup.

A recent study on a 96-hour Roundup exposure to the economically important tropical fish tambaqui found disturbed gill morphology, inhibited cholinesterase activity in the brain and DNA damage in erythrocytes [41]. They found a sixfold increase in a genetic damage indicator (GDI) in erythrocytes, using the “comet” assay method. Similarly, the comet assay applied to goldfish erythrocytes revealed DNA damage following exposure to glyphosate [42], and studies on exposure of eels to realistic concentrations of Roundup and the principal individual components, glyphosate and the surfactant polyethoxylated amine (POEA) in isolation, confirmed DNA damage in erythrocytes [43, 44].

Folate deficiency mimics radiation in damaging DNA through single- and double-strand breaks as well as oxidative lesions [45]. It is estimated that 10% of the US population is at risk from folate deficiency-induced DNA damage. Cancer of the colorectum in particular is linked to folate deficiency [45, 34], which causes reduced bioavailability of cytosine methylation capacity in DNA, inappropriately activating proto-oncogenes and inducing malignant transformation. Folate is also itself crucial for DNA synthesis and repair. Folate deficiency can also lead to uracil misincorporation into DNA and subsequent chromosome breaks [34]. Folate is an essential B vitamin, but it can be synthesized by gut microbes, particularly *Lactobacillus* and *Bifidobacterium* [46]. Glyphosate is a patented *antimicrobial agent*, and these two species are more vulnerable than others to growth inhibition by glyphosate [47]. Furthermore, folate is derived from chorismate, a product of the shikimate pathway that glyphosate disrupts [48].

## 7. SUCCINATE DEHYDROGENASE INHIBITION

A study on *Escherichia coli* revealed that glyphosate suppressed three different components of the succinate

dehydrogenase (SDH) enzyme, cytochrome b556, the avoprotein subunit and the hydrophobic subunit, reducing their activity three- to fourfold [48]. Roundup cytotoxicity in human cells is mediated in part through inhibition of SDH, a key enzyme in mitochondrial complex II [49–51]. A theoretical study of the mechanism of inhibition suggests that glyphosate binds at the succinate binding site with a higher binding energy than succinate, thus blocking substrate bioavailability [52]. Roundup has also been shown to depress complexes II and III [53].

Both SDH (complex II) and fumarate hydratase (FH) (complex III) are tumour suppressors. Their suppressive mechanism can be understood through the effects of enhanced glycolysis following their inhibition [54]. Mutations in SDH lead to the development of paraganglioma (tumours originating in the ganglia of the sympathetic nervous system), and pheochromocytoma (neuroendocrine tumours of the adrenal glands), and mutations in FH cause renal cell carcinoma. Neuroblastoma is the most common extracranial solid tumour in infants and young children [55]. An increase in growth rate and invasiveness in neuroblastomas is linked to impaired succinate dehydrogenase function [56].

Succinate and fumarate will accumulate in mitochondria when SDH and/or FH are suppressed, and they leak out into the cytosol. Two newly recognized signalling pathways result in enhanced glycolysis in a “pseudohypoxic response”, as well as resistance to apoptotic signals [54]. A characteristic feature of tumour cells is their increased use of glycolysis as a source of energy, even in the presence of available oxygen, a phenomenon referred to as the Warburg effect [57, 58]. Malignant, rapidly growing tumour cells typically have glycolytic rates up to 200 times higher than those of their normal tissues of origin, even when oxygen is plentiful.

## 8. GLYOXAL, METHYLGLYOXAL AND GLYOXYLATE

In this section, we discuss the potent toxicity of multiple metabolites of fructose that are plausibly present in foods derived from glyphosate-resistant crops, or as a contaminant in glyphosate-based products, or as a breakdown product generated endogenously following glyphosate exposure. These include glyoxylate, glyoxal and methylglyoxal. We show that these molecules are genotoxic and can induce cancer. We surmise that their toxicity is enhanced by glyphosate exposure diminishing bioavailability of vitamin E, an antioxidant.

Vitamin E, a tocopherol, is derived from the shikimate pathway, which glyphosate disrupts [59]. One of the best characterized functions of tocopherols is to protect biological membranes against oxidative stress. Superoxide dismutase (SOD) catalyses the conversion of superoxide anion



( $O_2^-$ ), a reactive oxygen species (ROS), to hydrogen peroxide ( $H_2O_2$ ) and molecular oxygen ( $O_2$ ). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can also produce ROS, which leads to proteinuria and haematuria [60].  $H_2O_2$  induces haem degradation in red blood cells, particularly when glutathione is deficient [61]. ROS causes irreversible DNA impairment, damage to lipid membranes and promotes the toxic carbonyl, malondialdehyde [62, 63]. Excessive lipid peroxidation induced with ingestion of glyphosate residues likely leads to an overload of maternal and foetal antioxidant defence systems following liver damage, as shown in rat studies by Beuret et al. [64].

Glyoxal and methylglyoxal are very potent glycation agents, considerably more reactive than either glucose or fructose [65, 66]. They attack the amine groups in amino acids, peptides and proteins to form advanced glycation end products (AGEs), and they cause carbonyl stress in the presence of oxidizing agents such as  $O_2^-$  and  $H_2O_2$  [66]. A study linking AGEs to cancer showed that methylglyoxal–bovine serum albumin (methylglyoxal-BSA) induced significant DNA damage [67]. Cancer incidence is increased in association with chronic renal failure, and this is likely due to the binding of AGEs to receptors for advanced glycation end products (RAGE), leading to increased intracellular formation of ROS [67].

Extremely high levels of methylglyoxal are found in commercial carbonated beverages sweetened with high fructose corn syrup (HFCS), but not in those that are sweetened with artificial sweeteners [68]. Since HFCS is derived from glyphosate-resistant corn, it is conceivable that the methylglyoxal was produced in the plant in response to glyphosate exposure. There is a plausible biological mechanism for this, caused by the accumulation of excessive amounts of phosphoenolpyruvate (PEP) as a consequence of the disruption of the enzyme, 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, that uses PEP as substrate for the first step in the shikimate pathway [69]. PEP suppresses glycolysis by binding to the active site in the enzyme, triose phosphate isomerase (TPI) [70], outcompeting the natural substrates.

Furthermore, PEP reacts with fructose to initiate its conversion to triose phosphate, also known as glyceraldehyde 3-phosphate (glyceraldehyde 3-p), as illustrated in Fig. 1 [71]. Glyceraldehyde 3-p is highly unstable and it spontaneously breaks down to methylglyoxal [72]. Severe impairment of TPI due to genetic defects leads to sharp increases in methylglyoxal and protein glycation, as well as oxidation and nitrosation damage [73]. Inhibition of glycolysis will increase the residence time of glyceraldehyde 3-p and increase its chances to spontaneously degrade to methylglyoxal. It

can be expected that similar problems will occur in gut microbes exposed to glyphosate, as well as human cells, and this may explain the increased levels of methylglyoxal observed in association with diabetes [74].

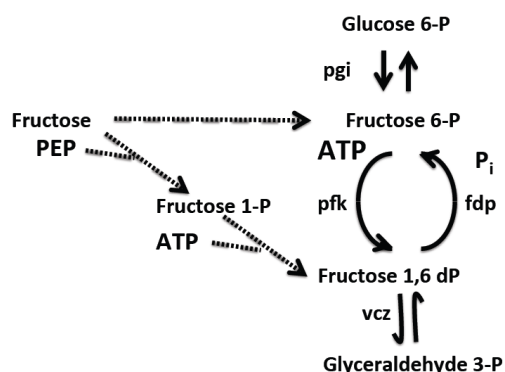


Figure 1. Possible pathways of fructose metabolism in *E. coli*. Genes are pgi, phosphoglucose isomerase; pc, fructose B-phosphate kinase; fdp, fructose diphosphatase; and fda, fructose diphosphate aldolase. PEP, phosphoenolpyruvate. Adapted from Fraenkel, 1968 [71].

A study comparing rats fed a high-fructose compared to a high-glucose diet revealed that those rats fed fructose experienced a significant increase in body weight, liver mass and fat mass compared to the glucose-fed rats [75]. This was accompanied by a reduction in physical activity, although the total number of calories consumed remained equivalent. We suspect that this phenomenon may be largely due to the presence of glyphosate and methylglyoxal contamination in the fructose (which was likely derived from the GMO Roundup-Ready HFCS). A study exposing male Sprague Dawley rats to a high-fructose diet during an interval over a period of four months showed elevated serum levels of methylglyoxal, along with several indicators of diabetes and metabolic syndrome, including expression of RAGE, NF- $\kappa$ B, mediators of the renin angiotensin system and elevated blood pressure [76]. At physiological concentrations, methylglyoxal can modify plasmid DNA and cause mutations and abnormal gene expression [77].

Glyphosate formulations are trade secrets, but a 2006 Monsanto patent proposed using oxalic acid (oxalate) as an additive to increase the toxicity of glyphosate to weeds [78]. Oxalate inhibits pyruvate kinase and this leads to an elevation in PEP along with a reduction in production of lactate and pyruvate. The synthesis of PEP in rat livers exposed to 0.1 mM oxalate more than doubled [79], which likely induces excess exposure to methylglyoxal as discussed above, causing liver stress. The effects of oxalate would be synergistic with the effects of glyphosate inhibition of the shikimate pathway in gut microbes, which can be expected to also increase PEP levels, since PEP is substrate for the enzyme that glyphosate disrupts.

Several anaerobic bacteria, including *Oxalobacter formigenes*, *Eubacterium lentum*, *Enterococcus faecalis* and *Lactobacillus acidophilus* can metabolize oxalate in the gut [80]. However, both oxalate decarboxylase and oxalate oxidase, enzymes involved in oxalate metabolism, depend on manganese as a cofactor [81], and manganese is chelated by glyphosate, making it unavailable to gut microbes [82, 83].

Elevated serum glyoxylate has been found to be an early marker for diabetes risk [84]. The conversion of glyoxylate to oxalate by the enzyme lactate dehydrogenase is inhibited by oxalate [85, 86]. Hence glyoxylate, derived from glyphosate breakdown, can be expected to accumulate in the presence of excess oxalate. Glyoxylate can be derived from glyoxal, and both glyoxal and glyoxylate have been proposed as key reactants in the production of glyphosate, as described in multiple patents from the mid-1980s [87, 88]. Furthermore, glyphosate can itself be metabolized to AMPA and glyoxylate by microbial action along two distinct pathways, via glycine oxidase or via glyphosate oxidoreductase [89]. *In vitro* exposure of hepatocytes to glyoxal showed hepatotoxicity induced by lipid peroxidation, ROS, and collapsed mitochondrial membrane potential [90, 91].

LDH is also known to be involved in tumour metabolism. A Monsanto study conducted by Johnson on rabbits found that extremely high doses of glyphosate (5000 mg/kg) severely downregulated production of LDH, reducing values in both male and female animals, whereas a fivefold lower dose (1000 mg/kg) upregulated LDH similarly in both males and females compared to the experimental control [92]. Glyphosate was administered by dermal absorption to three groups, each of 5 male and 5 female rabbits. Doses of 100, 1000 and 5000 mg/kg were held in place by occlusion for 6 hours/day, five days/week for 21 days. A control group of the same numbers of animals and sex did not receive the compound. Results for the control, low-, mid- and high-dose groups were 250, 169, 291 and 76 for male animals and 189, 149, 258 and 28 for female animals, respectively. Not understanding glyphosate's nonmonotonic dose-response relationship caused Johnson to dismiss this haematological finding. A similar pattern of LDH regulation was recorded by Stout & Ruecker in 1990 in experiments with albino rats [15].

A Monsanto patent application from 1985 describes the invention as follows: "glyphosate and various glyphosate derivatives can be produced with very high selectivity by the reductive alkylation of aminomethylphosphonic acid, its salts or its esters, in an aqueous medium with a carbonyl compound, such as, for example, glyoxal, glyoxylic acid, a glyoxylate salt, or a glyoxylate polyacetal salt or ester"

[88]. An earlier US patent application disclosed a similar process whereby aminomethylphosphonic acid is reacted in an aqueous medium with glyoxal in the presence of sulfur dioxide to produce glyphosate. Methylglyoxal is cytotoxic, and it has been shown to arrest growth and react with nucleotides, increasing the incidence of sister chromatid exchanges, a step towards tumorigenesis [93].

Methylglyoxal also decreases protein thiols, especially glutathione, an essential antioxidant. In *in vitro* studies, glyphosate has also been shown to reduce glutathione levels in mammalian cells, possibly mediated through methylglyoxal [94]. Methylglyoxal induces DNA mutations mainly at G:C base pairs, and it severely inhibits DNA replication by inducing cross-links between DNA and DNA polymerase [95]. The mutagenicity of methylglyoxal is suppressed by sulfur-containing molecules, such as sulfite, cysteine and glutathione [96]. Glyphosate has been shown to deplete methionine levels by 50% to 65% in a glyphosate-sensitive carrot plant line [97]. Methionine is an essential sulfur-containing amino acid crucial for maintaining levels of cysteine, glutathione and sulfate. Most bacteria possess biosynthetic pathways for methionine [98], and it is possible that glyphosate disrupts their ability to supply this critical nutrient to the host.

Glyoxalase is a key enzyme in the pathway that detoxifies methylglyoxal. Mouse studies have demonstrated that its overexpression can reduce AGE production and oxidative damage associated with hyperglycaemia [99], thus demonstrating a direct link between methylglyoxal and these pathologies. Glyoxalase is upregulated in association with rapid cell proliferation [100] and also in association with some cancers, including gastric cancer [101] and prostate cancer [102] (gastric cancer is the second highest cause of cancer-related mortality worldwide [103]). Overexpression of glyoxalase I is associated with increased gastric wall invasion and lymph node metastasis [101]. Glyphosate exposure has been shown experimentally to induce increased expression of glyoxalase activity in *Arachis hypogaea* (groundnut), which was engineered to be glyphosate-tolerant [100]. In addition, the observed upregulation of redox-regulated kinases, phosphatases and transcription factors shows the importance of redox couples to reorganize growth and metabolic needs under stress conditions, such as exposure to glyphosate. Mitogen-activated protein kinase (MAPK) phosphatases (MKPs) play an important rôle in the development of cancer in humans [104].

## 9. N-NITROSOGLYPHOSATE AND N-NITROSOSARCOSINE

As was shown by Monsanto's own studies [26], glyphosate readily reacts with nitrogen oxides to form N-nitrosoglyphosate (NNG), which is of great concern due



to its toxicity [105]. N-nitroso compounds (NOCs) can induce cancer in multiple organs in at least 40 different animal species, including higher primates [106–108]. In *in vitro* studies on human liver slices, the mechanism of action was shown to be nucleic acid alkylation [109]. Schmahl and Habs commented: “N-nitroso compounds can act carcinogenically in a large number of animal species; there is no rational reason why human beings should be an exception, all the less so since *in vitro* experiments have shown N-nitroso compounds are metabolized in the same way by human livers as by the livers of experimental animals” [108, p. 240]. Several different nitrosylated compounds have been targeted as potential carcinogenic agents, although it is conceded that the long lag time between exposure and tumour development makes it difficult to recognize the links [110]. Dietary N-nitrosyl compounds especially are thought to increase the risk of colon cancer and rectal carcinoma [111, 112].

The Food and Agricultural Organization of the United Nations (FAO) has set a strict upper limit of 1 ppm NNG [113]. The accepted methodology for measuring contamination levels, proposed by Monsanto in 1986 [114], has complicated instrumentation and operation conditions and is relatively insensitive [105]. New advanced methodologies offer safer and more reliable testing methods [115, 105].

One of the pathways by which some bacteria break down glyphosate is by first using carbon-phosphorus lyase (C-P lyase) to produce sarcosine as an immediate breakdown product [89, 116]. Nitrosylated sarcosine is well recognized as a carcinogenic agent. Injection of 225 mg/kg of nitrososarcosine into mice at days 1, 4 and 7 of life led to the development of metastasizing liver carcinomas in later life in 8 out of 14 exposed animals [117].

Elevated levels of sarcosine are also linked to prostate cancer, particularly metastatic prostate cancer [118]. An unbiased metabolomic survey of prostate cancer patients identified elevated levels of serum or urinary sarcosine as a marker of aggressive disease [119] (prostate cancer is the most commonly diagnosed cancer in men in the USA, and it afflicts one in nine men over the age of 65 [120]). In both *in vitro* and *in vivo* prostate cancer models, exposure to sarcosine, but not glycine or alanine, induced invasion and intravasation [119].

## 10. IMPAIRED GLYCINE SYNTHESIS

Perhaps surprisingly, a recent study has proposed that glyphosate might serve a useful rôle in cancer treatment due to its ability to inhibit glycine synthesis [121]. Glycine is essential for the synthesis of DNA and, therefore, for cell proliferation. *In vitro* studies on 8 different cancer

cell lines (including prostate, ovarian, cervical and lung cancer) demonstrated that glyphosate at doses ranging from 15 to 50 mM was cytotoxic to tumour cells, and that cytotoxicity to normal cell lines required higher doses (e.g., 100 mM). It was hypothesized that the mechanism of action involved impaired glycine synthesis due to glyphosate acting as a glycine mimetic.

In direct contradiction, however, glycine has been shown to prevent tumorigenesis [122] and it is a potent anti-angiogenic nutrient that suppresses tumour growth, possibly through activation of a glycine-gated chloride channel [123]. Impaired glycine synthesis likely has other adverse effects as well, such as the possibility that glyphosate interferes with glycine conjugation of benzene-based compounds. In particular, this is a mechanism used by gut microbes, particularly *Bifidobacteria*, to detoxify phenolic compounds, producing hippurate (benzoylglycine), a glycine conjugate of benzoic acid, as a mechanism for detoxification [124]. Glycine has been shown to be a limiting factor for hippurate production [125]. We stated earlier that glyphosate preferentially harms *Bifidobacteria* [46], and studies have shown reduced counts of *Bifidobacteria* in obese rats along with reduced excretion of hippurate [126]. Obese humans have also been shown to have reduced urinary hippurate [127]. Furthermore, lower urinary hippurate is linked to ulcerative colitis, particularly Crohn’s disease [128]. A Swedish study of over 21 000 Crohn’s disease patients identified increased risk of a broad range of cancers, including liver, pancreatic, lung, prostate, testicular, kidney, squamous cell skin cancer, nonthyroid endocrine tumours and leukaemia [129]. Crohn’s and inflammatory bowel disease have been increasing in incidence in the USA in step with the increase in glyphosate usage on corn and soy crops ( $R = 0.938, P \leq 7.1 \times 10^{-8}$ ) [1].

## 11. COLON AND LIVER CANCER

As shown in Table 1, the incidence of liver cancer in the USA has increased substantially in the past two decades, *pari passu* with the increase in glyphosate usage on corn and soy crops ( $P \leq 4.6 \times 10^{-8}$ ).

Nonalcoholic steatohepatitis (NASH) is a fatty liver disease that has been linked to excess dietary fructose [130]. We hypothesize that it is due primarily to the disruption in gut metabolism of fructose due to glyphosate blocking the shikimate pathway, as discussed previously. Fructose, which should have been processed in the gut leading to production of aromatic amino acids, instead is delivered to the liver, which converts it into fat for either local storage or distribution within low-density lipid particles (LDL). NASH affects a large proportion of the US population and is increasing in prevalence worldwide

with adoption of a “Western diet” [131]. NASH causes cirrhosis and increases risk of liver cancer [131, 132]. Hepatocellular carcinoma (HCC) is the most common cause of obesity-related cancer deaths among middle-aged men in America. The consumption of refined carbohydrates in soft drinks has been postulated to be a key factor in the development of NASH [130]. As we have seen, soft drinks containing HFCS are very high in methylglyoxal.

A study from 1988 on children with severe chronic liver disease revealed that those children with low vitamin E levels were susceptible to H<sub>2</sub>O<sub>2</sub>-induced haemolytic anaemia [133]. We earlier discussed the rôle of glyphosate in depleting vitamin E. Haemolysis leads to haemochromatosis (release of free iron from haem). The endocrine glands, heart, liver, testes and pancreas are all affected by haemochromatosis. Damage to pancreatic islet  $\beta$ -cells from iron deposition can lead to cellular death and functional impairment associated with diabetes [134]. Other effects of haemochromatosis include bone and joint pain, arthritis, cardiomyopathy and testicular problems.

The liver synthesizes substantial amounts of haem, which is needed primarily for the cytochrome P450 (CYP) enzymes, which perform many important rôles, including bile acid synthesis, hormone activation and breakdown, and detoxifying many carcinogenic agents, including phenolic and other organic xenobiotics as well as drugs and bilirubin. Glyphosate likely contributes to the destruction of CYP enzymes both through H<sub>2</sub>O<sub>2</sub> attack at their haem centre as well as through direct interference via nitrosylation at the active site by glyphosate [11]. CYP-mediated drug metabolism is impaired in patients with liver disease, particularly CYP1A, CYP2C19, and CYP3A [135], and this makes these individuals even more susceptible to liver damage.

Inflammation and metabolic disorders are intimately linked, and both are characteristic features of diabetes and obesity [136]. Diabetes and obesity are linked to dramatically higher risk of cancer, particularly of the liver and gastrointestinal tract [137]. This is directly linked to bile acid dysregulation and dysbiosis of the gut microbiome. Elevated levels of cytotoxic secondary bile acids and inflammation induced by an immune response to gut pathogens induce heightened oxidative DNA damage, increased cell proliferation and enterohepatic carcinogenesis [137]. Temporal patterns of glyphosate use on corn and soy crops strongly correlate with the increase in both diabetes and liver cancer observed over the same time interval [1].

Gut dysbiosis, due in part to glyphosate’s antimicrobial effects, leads to gut inflammation and impairment of the gut barrier function. This means that pathogens will escape the gut and infiltrate the liver. Exposure to

endotoxin produced by gut microbes, such as lipopolysaccharides (LPS) leads to inflammation in the liver along with hepatic fibrosis [138]. Several types of chronic liver disease are associated with increased levels of bacterial LPS in the portal and/or systemic circulation [139].

Acute hepatic porphyrias are disorders caused by enzyme defects in haem biosynthesis [140], and they are risk factors for liver cancer [141–143]. Glyphosate has been shown to disrupt haem synthesis, by suppressing the enzyme that activates the first step, combining glycine with succinyl coenzyme A to form  $\delta$ -aminolevulinic acid [144]. An often-overlooked component of glyphosate’s toxicity to plants is inhibition of chlorophyll synthesis [145], as  $\delta$ -aminolevulinic acid is also a precursor to chlorophyll as well as haem.

$\gamma$ -glutamyl transferase (GGT) is a membrane-bound enzyme that decomposes glutathione into cysteinyl glycine and glutamate; it is highly expressed in the liver. Excess serum GGT has been linked to both oxidative stress [146] and increased cancer risk [147] as well as many other diseases [148]. In a study on 283 438 people who were divided into five subgroups based on GGT level, a hazard ratio of 18.5 for risk of hepatic carcinoma was ascertained for the highest level compared to the lowest [149]. Another study based in Korea found an increased risk of multiple cancers in association with elevated GGT: most especially liver cancer, but also cancer of the esophagus, larynx, stomach, bile ducts, lungs and colon [150]. GGT induces generation of reactive oxygen species through interactions of cysteinyl glycine with free iron [151, 152].

Exposure to Roundup at low doses increased GGT expression in rat testis and Sertoli cells [94]. A comparison between goats fed GM Roundup-Ready solvent-extracted soybean *vs* goats fed a conventional soy equivalent revealed that the male kids born to the goats fed the GM soy had elevated expression of GGT in both liver and kidney ( $P < 0.01$ ) [153]. A study has shown that 70% of GM Roundup-ready soy samples had significant levels of glyphosate, whereas the conventional soy did not [154].

Exposure of Wistar rats to the herbicide Glyphosate-Biocarb over a period of 75 days resulted in liver damage, including elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible hepatocyte damage, as well as large deposition of reticulin fibres containing collagen type III [155], suggesting liver fibrosis [156], which is a major risk factor for hepatocarcinogenesis.

Excessive retinoic acid signalling in the liver is expected due to the interference of glyphosate with liver CYP enzymes [11, 157, 158], because the CYP2C gene

family is needed to metabolize retinoic acid in the liver [159]. The action of retinoic acid is likely mediated through sonic hedgehog signalling [160]. Studies on mice have revealed that hedgehog signalling induces fibrosis and hepatocellular carcinoma [161]. Studies on tadpoles have demonstrated that glyphosate produces teratogenic effects characteristic of excessive retinoic acid signalling, and these effects were reversed by a retinoic acid antagonist [162].

## 12. PANCREATIC CANCER

Pancreatic cancer is one of the cancers whose incidence is going up in step with the increase in glyphosate usage on corn and soy crops ( $R = 0.918$ ;  $P \leq 4.6 \times 10^{-7}$ ) [1]. As of 2002, pancreatic adenocarcinoma was the fourth leading cause of cancer death in the USA, with an overall 5-year survival rate of less than 5% [163]. We have already noted that excess methylglyoxal exposure can lead to diabetes. Direct evidence of this was obtained when methylglyoxal injection into Sprague Dawley rats caused pancreatic  $\beta$ -cell dysfunction [164]. We earlier discussed the rôle of excess iron deposition in the destruction of pancreatic  $\beta$  cells [134].

Glyphosate's metal chelation effects led to severe manganese deficiency in cows [83]. Rats fed a diet deficient in manganese showed significantly lower concentrations of manganese in liver, kidney, heart and pancreas compared to controls [165]. Pancreatic insulin content was reduced by 63%, and insulin output was correspondingly reduced, suggesting that manganese deficiency may play a direct rôle in insulin-deficient diabetes and islet cell stress.

Acinar cell carcinoma is the second most common type of pancreatic cancer, characterized histologically by zymogen-like granules as well as fibrillary internal structures in the tumour cells [166]. A comparison between mice fed GM soy and wild soy demonstrated alterations in pancreatic acinar cells including smaller zymogen granules and less zymogen content in one month-old mice, along with reduced production of  $\alpha$ -amylase [167]. The authors did not consider possible effects of glyphosate contamination, even though another study has shown significant glyphosate residues in GM soy as compared to conventional soy treated with glyphosate [154]. Pancreatic atrophy of the acinar cells along with degranulation and intracellular fibrillation is a fundamental aspect of the childhood wasting disease kwashiorkor [168], which is linked to disrupted gut microbes [169], and may also be in part attributable to glyphosate poisoning.

A two-year study of glyphosate toxicity to rats reported by the EPA in 1991 showed several signs of tumours, which were ultimately dismissed partly because of a lack of a dose–response relationship, and in part because it was argued that historical controls (but not the controls in the study) demonstrated tumours at comparable rates, but under very different and uncontrolled dietary and lifestyle practices [170]. The most frequently observed tumours were pancreatic islet cell adenomas in males, thyroid C-cell adenomas and/or carcinomas in males and females, and hepatocellular adenomas and carcinomas in males. Both low-dose and high-dose, but not mid-dose, males had a statistically significant increased incidence of pancreatic islet cell adenomas.

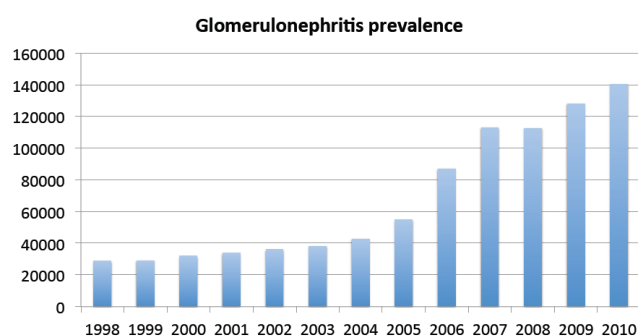


Figure 2. Incidence of nephritis and kidney failure reports in the US CDC's hospital discharge data from 1998 to 2010 normalized to counts per million population each year. This includes all reports of ICD-9 codes from 580 to 589.

## 13. KIDNEY CANCER

Chronic kidney disease (CKD) and cancer are closely linked in reciprocal fashion: cancer or its treatment can cause CKD and patients with CKD have increased risk of cancer. Dialysis patients have an increased risk ranging from 10% to 80%; kidney transplant recipients have a 3- to 4-fold increased risk of cancer [6]. The number of patients with kidney failure treated by dialysis and transplantation increased dramatically in the USA from 209 000 in 1991 to 472 000 in 2004 [171]. There have been concurrent increases in earlier stages of chronic kidney disease such as albuminuria and impaired glomerular filtration [172]. Since 2004, this trend has worsened. Figure 2 shows the trend over time in the US Centers for Disease Control (CDC)'s hospital discharge data<sup>4</sup> for ICD-9 codes 580-589, including acute and chronic glomerulonephritis, nephritis and nephropathy, acute and chronic renal failure, renal sclerosis, and disorders resulting from impaired renal function. There has been an alarming rise in the frequency of these conditions, especially since 2006.

<sup>4</sup> [ftp://ftp.cdc.gov/pub/Health Statistics/NCHS/Datasets/NHDS](ftp://ftp.cdc.gov/pub/Health%20Statistics/NCHS/Datasets/NHDS)



Studies on rats show that CYP 2B1 plays a pivotal rôle as an important site for ROS production through cytotoxicity in the glomeruli [173]. The breakdown of the CYP haem protein through attack by  $H_2O_2$  leads to the release of catalytic iron, which, in turn, generates more potent tissue-damaging oxidants such as the hydroxyl radical. Glyphosate's induction of excess  $H_2O_2$  as discussed earlier would cause an increase in the bioavailability of catalytic free iron to work synergistically with  $H_2O_2$  to cause toxicity.

Methylglyoxal and other glyating agents may be a significant factor in the development of kidney disease. Twelve weeks of administration through drinking water of methylglyoxal to Dahl salt-sensitive rats led to an increase in systolic blood pressure and significantly increased urinary albumin excretion, glomerular sclerosis, tubular injury, myocardial collagen content and cardiac perivascular fibrosis [174]. Renal markers of AGE production, oxidative stress and inflammation were all elevated.

Acquired cystic kidney disease (ACKD) can lead to renal tumours, and the tumours often accumulate calcium oxalate crystals [175]. These tumours are often associated with distinctive morphological features, where the tumour cells have ill-defined cell membranes, abundant granular eosinophilic cytoplasm, large nuclei and prominent nucleoli. In another study identifying intratumoral calcium oxalate crystal deposition in two cases of high-grade renal carcinomas, the authors suggested a relationship between tumour growth and oxalate crystal deposition [176]. This suggests a rôle for oxalic acid added to glyphosate-based formulations.

An *in vitro* study on rat testis and Sertoli cells demonstrated that Roundup triggers calcium-mediated cell death associated with reductions in levels of the antioxidant glutathione, along with thiobarbituric acid reactive species (TBARS) and protein carbonyls indicative of protein oxidation and glycation damage [94]. Administration of L-buthionine(S,R)-sulfoximine (BSO), a specific inhibitor of glutathione synthesis, to rats caused reduced glutathione levels in the kidneys and a marked increase in pathologies linked to polycystic kidney disease [177].

#### 14. CATARACTS AND MELANOMA

As we showed previously, Monsanto's own studies revealed increased risk of cataracts following exposure to Roundup. Early-onset cataracts are associated with insufficient antioxidative activity and, therefore, are a potential risk of cancer, as verified in a recent nationwide study based in Taiwan [178].

Methylglyoxal is implicated in cataract development [179, 180]. Methylglyoxal induces endoplasmic reticulum stress in human lens epithelial cells, and activates an

unfolded protein response leading to overproduction of ROS. Overexpression of Keap1 protein causes proteasomal degradation of Nrf2, thus suppressing Nrf2-dependent stress protection. As a consequence, the cellular redox balance is altered toward lens oxidation and cataract formation [179].

There is a link between cholestasis and cataracts via poor absorption of nutrients that protect the lens from UV damage. Studies on short-term exposure of catfish to sublethal levels of Roundup revealed toxicity to the gills, liver and kidneys [181]. The observed elevated levels of unconjugated bilirubin and alanine aminotransferase (ALT) are indicative of cholestasis, likely in part a consequence of impaired CYP enzyme function. Cholestasis impairs the absorption of fat-soluble vitamins and previtamins such as the carotenoids [182]. Lutein and zeaxanthin are carotenoids that play an important rôle in the lens and macular region of the retina to protect from oxidative damage due to sunlight exposure [183, 184]. They are highly lipophilic and, therefore, like the fat-soluble vitamins, depend on adequate bile flow for gastrointestinal absorption. Cholestatic patients have greatly reduced serum levels of these nutrients [182].

Tryptophan is a product of the shikimate pathway that glyphosate suppresses. A tryptophan-free diet induces cataracts in young Wistar rats, along with a significant decrease in lens weight and water-soluble lens protein [185]. Kynurenine is a breakdown product of tryptophan, and it has been suggested that kynurenine and its glycoside derivatives in the ocular lens protect the retina from UV light by absorbing UV radiation [186]. Kynurenine is present in excessive concentrations in cataracts [186].

Melanoma is one of the types of cancer that have been linked to glyphosate exposure in agriculture. An age-adjusted analysis revealed an 80% increased risk of melanoma associated with glyphosate use in a study on pesticide applicators in Iowa and North Carolina [187]. It is possible that impaired supply of the aromatic amino acids, tryptophan and tyrosine due to disruption of the shikimate pathway in gut microbes plays a rôle in increased risk to melanoma.

*In vitro*, exposure to 0.1 mM glyphosate induced hyperproliferation in human skin keratinocytes (HaCaT) cells, suggesting carcinogenic potential [188]. The mechanism involves increased ROS expression and the emptying of intracellular calcium stores, which facilitates basal cell or squamous cell carcinomas. Cells accumulated in S-phase of the cell cycle, while mitochondrial apoptotic signalling pathways were downregulated.

Melanin plays an important protective rôle in the skin against UV exposure, and dark-skinned races have

significantly reduced risk of skin melanoma because of their naturally higher levels of melanin [189]. Melanosomes are tissue-specific organelles in pigment cells that resemble lysosomes, in which melanin is synthesized and stored [190]. L-tyrosine is the precursor to melanin synthesis, and the pathway involves the intermediary, L-dopa. Both L-tyrosine and L-dopa, when supplied to cells with melanogenic potential, increase not only the synthesis of melanin but also the formation of melanosomes within the cells [191].

While blacks have protection against skin cancer due to the high concentration of melanin in their skin, dark skin also appears to be a risk factor for autism. A study based in Los Angeles showed that children born to black foreign-born women had a substantially increased risk for low-functioning autism [192]. A similar observation has been made in Sweden [193] and the UK [194]. One possibility is that increased demand for melanin in the skin depletes the supply of tyrosine for dopamine synthesis. Genetic mutations in dopamine transport proteins have been linked to autism [195, 196]. The defect features a persistent reverse transport of dopamine (substrate efflux from the synapse), which reduces the amount of time extracellular dopamine is available for signalling effects [195]. Other genes of the dopaminergic network are also linked to autism, including syntaxin [197] and enzymes involved in dopamine metabolism [198]. Hence, we hypothesize that reduced bioavailability of tyrosine (due to disruption of the shikimate pathway in gut microbes) for either dopamine synthesis or melanin synthesis leads to different outcomes (autism vs melanoma) depending on race-related skin colour.

Tryptophan is an essential amino acid for lymphocyte activation and proliferation, which promotes surveillance and elimination of tumour cells [199, 200]. Tryptophan is also produced by gut microbes via the shikimate pathway that glyphosate disrupts, suggesting that glyphosate exposure to gut microbes could impair tryptophan bioavailability to the human host. The enzyme indoleamine 2,3-dioxygenase (IDO) catalyses the degradation of tryptophan to kynurenines. Tumours of the lung [201], colon [202], liver [203], breast [204] and uvea [205], as well as skin melanoma [206], overexpress IDO, and it is believed that this leads to an ability to evade immune surveillance by T-cells via depletion of tryptophan bioavailability in the surrounding milieu [205]. It is interesting that IDO offers significant protection from UV damage by producing tryptophan-based filters that protect the cornea, lens and retina from UV-induced photo-oxidation [207, 208]. It may well be that tumours exploit IDO for this purpose as well. Clearly, decreased bioavailability of tryptophan due to glyphosate's effects on gut microbes would enhance

the tumour's ability to deplete tryptophan and avoid immune surveillance, but might also lead to accelerated DNA damage within the tumour and increased risk of metastasis [209].

## 15. THYROID CANCER

The incidence of thyroid cancer in the United States has increased dramatically in the past two decades, in step with the increase in glyphosate usage on corn and soy crops ( $R = 0.988$ ,  $P \leq 7.6 \times 10^{-9}$ ) [1]. It is not clear how glyphosate might increase risk of thyroid cancer beyond the general factors already described previously in this paper, but it is possible that impaired selenium incorporation into selenoproteins plays a rôle.

Selenium is an important trace element involved in the protection of cells from oxidative stress, and it is particularly important for the thyroid. Low serum levels of selenium are associated with increased risk of thyroid cancer, and probably play a rôle in carcinogenesis. All three of the deiodinases that convert thyroxine (T4) into triiodothyronine (T3) contain selenocysteine, as do glutathione peroxidase and thioredoxin reductase, which are important antioxidant enzymes essential for protecting thyrocytes from oxidative damage [210].

The microbiome plays an important rôle in incorporating free selenium into selenoproteins, especially selenocysteine. *Lactobacillus reuteri* is a popular species in probiotics, shown to be effective against diarrhoea in children [211], and to inhibit the prooxidant cytokine TNF- $\alpha$  in humans [212]. This species has been found to be especially effective in its ability to produce selenocysteine, and has been proposed to have therapeutic benefit in cases of selenium deficiency [213]. *Lactobacillus* is especially vulnerable to glyphosate due to its crucial and unusual need for manganese as an antioxidant [214, 215], so it is plausible that diminished *Lactobacillus* representation in the gut could lead to an impaired supply of selenocysteine for the thyroid.

## 16. BREAST CANCER

Breast cancer accounts for one third of cancer diagnoses and 15% of cancer deaths in women in the United States. As mentioned previously, an *in vitro* study has confirmed that glyphosate stimulates proliferation of human breast cancer cells when present in concentrations of parts per trillion [9].<sup>1</sup> This effect is specific to hormone-dependent cell lines, and is mediated by the ability of glyphosate to act as an oestrogenic agent.

One can obtain an estimate of the time trends in breast cancer by looking at the CDC's hospital discharge data. The results show a steady decrease in breast cancer



diagnoses up to 2006, followed by an increase from 2006 to 2010 (the last year for which data are available). The decrease can logically be explained by a growing awareness of the increased risk of breast cancer associated with hormone replacement therapy (HRT). A Women's Health Initiative (WHI) study, published in 2003, showed a 24% increase in invasive breast cancer risk associated with oestrogen/progestin therapy [216]. In direct response to this alarming report, HRT prescriptions in the United States decreased by 38% in 2003. A large study on 1 642 824 women published in 2013, based on the Breast Cancer Surveillance Consortium, revealed that HRT (commonly used to treat symptoms of menopause) increased the risk of breast cancer by 20% in whites, Asians and hispanics, but not in blacks [217].

By forming separate records from the hospital discharge data for black and white women, it can be confirmed that the breast cancer rates among blacks remained flat up to 2006, supporting the observation that black women are not subject to increased risk from HRT. This suggests that one can build a model to correct for the influence of reduced use of HRT among white women in order to arrive at a time trend that might more closely capture any effects of glyphosate. A simple decaying exponential model matches well for the Caucasian data from 1998 to 2006, and this model can be extended into the time interval from 2006 to 2010, and then subtracted from the original plot, to yield a plot of the residual trends for breast cancer. The resulting plot is shown in Fig. 3 alongside rates of glyphosate usage on corn and soy crops. The correlation coefficient is 0.9375 ( $P$ -value  $\leq 0.0001132$ ).

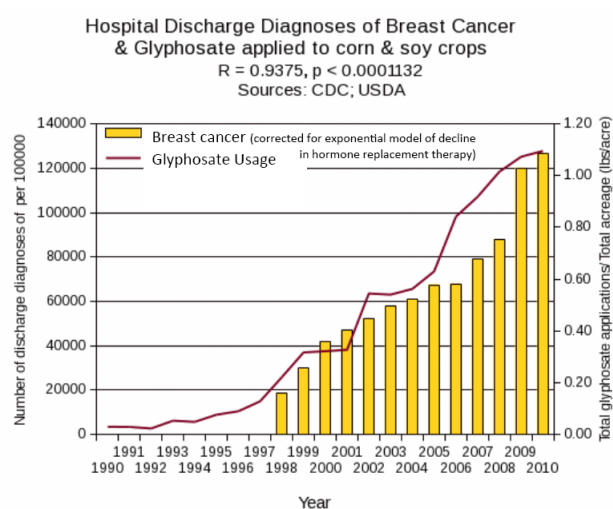


Figure 3. Incidence of breast cancer in US hospital discharge data from 1998 to 2010 normalized to counts per 1,000,000 population each year, after subtraction of an exponential model accounting for the decline in the years up to 2006 in the Caucasian subpopulation [see text]. This includes all reports of ICD-9 codes 174 and 175. The red line shows trends in glyphosate usage on corn and soy crops over the same time period.

A study on rats conducted by Séralini et al. [7] divided the rats into four groups: (1) control, (2) GM maize without Roundup, (3) GM maize with Roundup, and (4) Roundup alone. The major tumours detected in the female rats were mammary fibroadenomas and adenocarcinomas. These authors summarized their findings as: “The Roundup treatment groups showed the greatest rates of tumour incidence, with 80% of animals affected with up to 3 tumours for one female, in each group.” For the group that received Roundup in their drinking water, all but one of the females presented with mammary hypertrophies and hyperplasias. The one exception suffered from a metastatic ovarian carcinoma.

Glyphosate may also indirectly increase risk of breast cancer by impairing metabolism of toxic phenolic compounds such as nonylphenols, diethylstilbestrol (DES), and Bisphenol A (BPA), all widely recognized to possess oestrogenic activity. Nonylphenols, also known as alkylphenols, are a family of organic compounds used extensively as additives in laundry detergents, lubricating oils, paints, pesticides, personal care products and plastics, which are known to be xenoestrogenic [218]. DES is an oestrogenic compound linked to vaginal tumours in women exposed *in utero* to this compound when it was mistakenly believed to be of therapeutic benefit. BPA, commonly used in plastics production, is now widely recognized as an endocrine disruptor. PCBs were widely used as coolants and insulating fluids for transformers and capacitors until their ban in 1979 by the US government due to recognition of their toxicity due to oestrogenic activity. However, they degrade very slowly, and therefore are still environmental pollutants today.

Liver CYP enzymes play an important rôle in metabolizing all of these xenoestrogenic compounds. CYP1A1 is upregulated in response to PCB exposure, and therefore it likely metabolizes these toxic phenols [219]. High serum levels of PCBs in conjunction with at least one (defective) exon 7 variant allele of CYP1A1 increased breast cancer risk [219]. CYP enzymes are also involved with the metabolism of nonylphenols [220]. Similarly, BPA is mainly metabolized by the CYP2C subfamily in the liver [221]. Thus, impaired CYP function due to glyphosate exposure [11, 157, 158] can be expected to interfere with metabolism of PCBs and therefore increase their oestrogenic potential, leading indirectly to increased risk of breast cancer.

High dietary iron enhances the incidence of carcinogen-induced mammary cancer in rats and oestrogen-induced kidney tumours in hamsters [222]. Oestrogen facilitates iron uptake by cells in culture. Elevated body iron storage increases the risk of several cancers, including breast cancer in humans. Although it might be argued that

glyphosate's chelating effects may protect from iron overload, glyphosate could increase the bioavailability of free iron due to its damaging effects on red blood cells [42, 223] working synergistically with its interference in haem synthesis [144], and by acting as an oestrogen mimetic to enhance iron uptake. Haem degradation by reactive oxygen species [224] will lead to the release of free iron, and we have previously discussed how glyphosate would induce oxidative stress. In fact, recent evidence strongly suggests that GGT induces lipid peroxidation of red blood cell membranes leading to haemolysis and the release of free iron from chelating agents [225]. This also results in impaired deformability which impedes their passage through narrow capillaries. GGT was found to be enhanced up to 5.4-fold in the liver in Séralini et al.'s long-term study of rats exposed to GMO's plus Roundup [7].

### 17. NON-HODGKIN'S LYMPHOMA

Striking increases in the incidence of non-Hodgkin lymphoma (NHL) cancer have occurred over the past three decades, both in Europe [226] and America [227]. Agricultural workers have a higher risk of NHL than the general population, but it is difficult to tease out the effects of glyphosate compared to the myriad other toxic chemicals they are exposed to, which also confer increased risk [228]. However, some studies have been able to directly link glyphosate to NHL. A threefold increased risk of NHL in association with glyphosate exposure was found in a 2002 study from Sweden [229]. A later Swedish study in 2008 of over 900 cancer cases also found a significant increased risk of NHL (OR 2.02) [230]. A Canadian study demonstrated a correlation between the number of days per year of glyphosate exposure and the risk of NHL [231].

Increased exposure to superoxide is implicated as a causal agent in oncogenesis [232], and manganese SOD (Mn-SOD) is an important antioxidant defence agent in mitochondria [233]. Mice engineered to be defective in Mn-SOD had increased DNA damage and higher cancer incidence [234]. We mentioned earlier that Mn-SOD is protective against pancreatic cancer. Mn-SOD expression was also found to be anomalously low in erythrocytes of patients suffering from NHL [235]. *In vitro* studies have shown that an Mn-SOD mimetic had an anti-proliferation effect on human NHL Raji cells [236]. Glyphosate's chelating effects on manganese can be expected to interfere with Mn-SOD function [82]. Increased Mn-SOD expression potentiates apoptosis of tumour cells exposed to dexamethasone [237]. Cationic manganese porphyrins, probably by acting as Mn-SOD mimetics, have also been found to play a protective rôle in treating NHL [238, 239].

Bone marrow involvement is common in NHL and, particularly for those of T-cell origin, it portends a poor prognosis [240]. An unpublished study by Monsanto in 1983 confirmed that glyphosate administered by intraperitoneal injection to rats reaches the bone marrow within 30 minutes [241]. In an experiment to assess potential toxicity to bone marrow cells [242], a single intraperitoneal dose of glyphosate at concentrations of 25 and 50 mg/kg was administered to Swiss albino mice. Chromosomal aberrations and micronuclei, analysed 24, 48, and 72 hours later, were shown to be significantly increased compared to vehicle control ( $P < 0.05$ ). Mitosis rates were also decreased, indicating cytotoxic effects.

Multiple myeloma is the second most common haematological malignancy in the USA after non-Hodgkin lymphoma; it constitutes 1% of all cancers [243]. In a prospective cohort study of 57 311 licensed pesticide applicators in Iowa and North Carolina, a greater than twofold increased risk of multiple myeloma was associated with ever-use of glyphosate [187].

Coeliac disease, along with the more general condition, gluten intolerance, has recently reached epidemic levels in the United States, and it has been hypothesized that this heightened wheat sensitivity is a direct consequence of glyphosate contamination of the wheat, due to the increasingly common practice of wheat desiccation with glyphosate just before harvest [158]. Coeliac disease patients are at increased risk of cancer, particularly non-Hodgkin lymphoma, and they have statistically a shortened lifespan mainly due to this increased cancer risk.

For coeliac disease patients, serum prolactin (PRL) levels are high in association with an unrestricted gluten-containing diet, and PRL has been proposed as a useful marker for coeliac disease [244]. PRL is an important regulatory hormone released by the pituitary gland, which is best known for inducing lactation. Bisphenol A, a well-established oestrogenic agent, has been shown to lead to hyperprolactinaemia and growth of prolactin-producing pituitary cells [245]. Prolonged exposure to Bisphenol A during childhood may contribute to the growth of a prolactinoma, the most common form of cancer of the pituitary. Oestrogen treatment of ovariectomized rats induced a marked elevation of serum PRL levels [246], and this was found to be due to oestrogen's ability to reduce the capacity of PRL cells to incorporate dopamine into their secretory granules. Since glyphosate has been confirmed to be oestrogenic, it is plausible that glyphosate contamination in wheat is the true source of the observed elevation of PRL in association with gluten ingestion among coeliac patients.

## 18. CONCLUSION

In this paper, we have reviewed the research literature on glyphosate and on the biological processes associated with cancer, and we have provided strong evidence that glyphosate is likely contributing to the increased prevalence of multiple types of cancer in humans. Monsanto's own early studies revealed some trends in animal models that should not have been ignored. Forty years of glyphosate exposure have provided a living laboratory where humans are the guinea pigs and the outcomes are alarmingly apparent.

We have shown that glyphosate transforms exposed cells into a tumour-provoking state by suppressing crucial enzymes in the electron transport chain, such as succinate dehydrogenase and fumarate hydratase. Glyphosate chelates manganese, reducing its bioavailability, and manganese is an important catalyst for Mn-SOD, which protects mitochondria from oxidative damage, which can cause mutations in DNA. Glyphosate also causes impaired metabolism of fructose, due to the accumulation of PEP following blockage of the shikimate pathway. This leads to the synthesis of multiple short-chain sugars that are known to be highly potent glycoating agents, such as methylglyoxal and glyoxalate. Glyphosate is readily nitrosylated, and nitrosyl glyphosate is known to be extremely toxic and carcinogenic. Microbial pathways convert glyphosate into sarcosine, a known marker for prostate cancer, likely due to its nitrosylated form.

An often overlooked aspect of glyphosate's toxicity is its interference with enzymes that have glycine as substrate, due to mimicry. Phenolic compounds are detoxified by gut microbes through glycine conjugation to produce products such as hippurate. Bifidobacteria are important for the rôle they play in protecting from these xenobiotics through such conjugation. Reduced hippurate is linked to Crohn's diseases and inflammatory bowel disease, which show epidemiological trends that match the increased use of glyphosate on core crops, and which are linked to increased risk of a broad range of cancers, most especially non-Hodgkin lymphoma. Lymphoma has also been linked to glyphosate through studies of environmental exposure in agricultural settings.

Multiple studies, both *in vitro* and *in vivo*, have shown that glyphosate damages DNA, a direct step towards tumorigenicity. These studies have been conducted on sea urchins, fish, mice and various human cell types *in vitro*. Children in Malaysia living near rice paddies have evidence of DNA damage.

Epidemiological studies strongly support links between glyphosate and multiple cancers, with extremely well matched upward trends in multiple forms of cancer in step with the increased use of glyphosate on corn and

soy crops. While these strong correlations cannot prove causality, the biological evidence is strong to support mechanisms that are likely in play, which can explain the observed correlations through plausible scientific arguments.

Glyphosate's links to specific cancer types can often be explained through specific pathologies. For example, succinate dehydrogenase deficiency is linked to adrenal cancer [17]. Selenoprotein deficiency is likely contributory towards thyroid cancer. Glyphosate's action as an oestrogen mimetic explains increased breast cancer risk. Prostate cancer is linked to sarcosine, a by-product of glyphosate breakdown by gut microbes. Impaired fructose metabolism links to fatty liver disease, which is a risk factor for hepatic tumorigenesis. Impaired melanin synthesis by melanocytes due to deficiencies in the precursor, tyrosine, a product of the shikimate pathway, can explain increased incidence of skin melanoma. This is compounded by tryptophan deficiency, as tryptophan is also protective against UV exposure.

Manganese deficiency stresses the pancreas and impairs insulin synthesis, and this could explain the recent epidemic in pancreatic cancer. Increased oxalate, due in part to the proprietary formulations, stresses the kidney and contributes to risk of renal tumours. Glyphosate's accumulation in bone marrow can be expected to disrupt the maturation process of lymphocytes from stem cell precursors. Glycine forms conjugates with organic benzene-derived carcinogenic agents, and glyphosate likely interferes with this process. Glyphosate's interference with CYP enzyme function impairs detoxification of multiple other carcinogenic agents, increasing their carcinogenic potential. Overall, the evidence of the carcinogenicity of glyphosate is compelling and multifactorial.

## APPENDIX: NEOPLASTIC INCIDENCE DATA FROM MONSANTO

### Two-Year Animal Studies

In this section we present selected tables tabulating tumours and malignancies, separately for male and female rats, in the long-term study conducted by Lankas & Hogan and reported on in an unpublished document in 1981 [17]. The rats were exposed to three different doses of glyphosate added to their feed (3, 10, and 30 mg kg<sup>-1</sup> day<sup>-1</sup>) and compared with unexposed controls.

Similarly, we present tables tabulating all of the tumours and malignancies that were found, separately for male and female mice, in the long-term study conducted by Knezevich & Hogan and reported on in an unpublished document in 1983 [18]. The mice were exposed to three different doses of glyphosate added to their feed (1000, 5000 and 30 000 ppm) and compared with unexposed controls.

Table A1. Incidence of neoplastic findings in male rats with glyphosate administered by diet. Part I. Data extracted from Lankas &amp; Hogan (1981) [17].

Glyphosate /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
		PITUITARY		
Adenoma	16/48 (33%)	19/49 (38%)	20/48 (40%)	18/47 (36%)
Carcinoma	3/48 (6%)	2/49 (4%)	3/48 (6%)	1/47 (2%)
		BRAIN		
Glioma	1/49 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
		HEART		
Reticulum cell sarcoma	0/49 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
		LUNG		
Sarcoma 0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (2%)	1/50 (2%)
Reticulum cell sarcoma	1/50 (2%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
MS <sup>a</sup> Malignant mixed tumour	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
		MANDIBULAR SALIVARY GLAND		
Reticulum cell sarcoma	0/49 (0%)	0/49 (0%)	1/49 (2%)	0/49 (0%)
		MEDIASTINAL LYMPH NODE		
MS <sup>a</sup> Fibrosarcoma	0/39 (0%)	0/39 (0%)	1/32 (3%)	0/35 (0%)
Reticulum cell sarcoma	1/39 (3%)	0/39 (0%)	1/32 (3%)	0/35 (0%)
		SPLEEN		
Reticulum cell sarcoma	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
		STOMACH		
Squamous cell carcinoma, Cardia	0/50 (0%)	0/49 (0%)	0/48 (0%)	1/49 (2%)
		JEJUNUM		
Reticulum cell sarcoma	0/49 (0%)	0/46 (0%)	1/48 (2%)	0/49 (0%)
		KIDNEY		
Tubular adenoma	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Reticulum cell sarcoma	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Lipoma	1/50 (2%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
		TESTES		
Interstitial cell tumour	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)

<sup>a</sup>MS = metastatic.



Table A2. Incidence of neoplastic findings in male rats with glyphosate administered by diet. Part II. Data extracted from Lankas & Hogan (1981) [17].

Glyphosate /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
	PROSTATE			
Reticulum cell sarcoma	0/50 (0%)	0/47 (0%)	1/49 (2%)	0/49 (0%)
	URINARY BLADDER			
Papilloma	0/46 (0%)	1/45 (2%)	0/43 (0%)	0/46 (0%)
	THYROID			
C-cell carcinoma	0/47 (0%)	0/49 (0%)	1/49 (2%)	0/49 (0%)
Follicular adenoma	1/47 (2%)	2/49 (4%)	4/49 (8%)	4/49 (8%)
	PARATHYROID			
Adenoma	0/27 (0%)	2/30 (4%)	0/28 (0%)	0/27 (0%)
	ADRENAL			
Reticulum cell sarcoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Pheochromo-cytoma	8/50 (16%)	8/50 (16%)	5/50 (10%)	11/50 (22%)
Cortical adenoma	2/50 (4%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
	SKIN			
Basosquamous cell tumour	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/49 (2%)
Sebaceous gland adenoma	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/49 (2%)
	PERIOcular TISSUE			
Squamous cell carcinoma	0/0 (0%)	0/0 (0%)	1/1 (100%)	0/0 (0%)
	SUBCUTANEOUS TISSUE			
Fibrosarcoma	2/10 (20%)	1/12 (8%)	2/10 (20%)	3/7 (43%)
Fibroma	0/10 (0%)	3/12 (24%)	1/10 (10%)	2/7 (29%)
Neuro brosarcoma	0/10 (0%)	0/12 (0%)	0/10 (0%)	1/7 (14%)
Lipoma	1/10 (10%)	2/12 (17%)	0/10 (0%)	0/7 (0%)
Osteogenic sarcoma	0/10 (0%)	0/12 (0%)	1/10 (10%)	0/7 (0%)
Malignant mixed tumour	0/10 (0%)	1/12 (8%)	0/10 (0%)	0/7 (0%)
	MEDIAS TINAL TISSUE			
Reticulum cell sarcoma	0/7 (0%)	0/1 (0%)	0/4 (0%)	1/2 (50%)
	ABDOMEN			
Lipoma	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/1 (100%)
	ABDOMINAL CAVITY			
Reticulum cell sarcoma	0/0 (0%)	0/0 (0%)	1/1 (100%)	0/0 (0%)
	LUMBAR LYMPH NODE			
MS <sup>a</sup> Islet cell carcinoma	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/1 (100%)
	SACRAL LYMPH NODE			
Reticulum cell sarcoma	0/1 (0%)	1/3 (33%)	0/3 (0%)	0/3 (0%)

<sup>a</sup>MS = metastatic.



Table A3. Incidence of neoplastic findings in female rats with glyphosate administered by diet. Part I. Data extracted from Lankas &amp; Hogan (1981) [17].

Glyphosate /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
PITUITARY				
Carcinoma	8/48 (17%)	7/48 (15%)	5/50 (10%)	12/49 (24%)
BRAIN				
Invasive pituitary carcinoma	0/50 (0%)	0/49 (0%)	1/50 (2%)	1/50 (2%)
Malignant lymphoma	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
Glioma	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
CERVICAL SPINAL CORD				
Malignant lymphoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
HEART				
Malignant lymphoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
LUNG				
Reticulum cell sarcoma	2/49 (4%)	2/50 (4%)	1/49 (2%)	3/50 (6%)
Malignant lymphoma	0/49 (0%)	1/50 (2%)	0/49 (0%)	1/50 (2%)
Adenocarcinoma	0/49 (0%)	0/50 (0%)	0/49 (0%)	1/50 (2%)
Carcinoma	0/49 (0%)	0/50 (0%)	1/49 (2%)	0/50 (0%)
LIVER				
Reticulum cell sarcoma	2/50 (4%)	2/50 (4%)	1/50 (2%)	2/50 (4%)
Malignant lymphoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Hepatocellular carcinoma	1/50 (2%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
MESENTERIC LYMPH NODE				
Malignant lymphoma	0/42 (0%)	0/39 (0%)	0/48 (0%)	1/47 (2%)
Reticulum cell sarcoma	0/42 (0%)	0/39 (0%)	0/48 (0%)	2/47 (4%)
PANCREAS				
Islet cell carcinoma	0/50 (0%)	1/50 (2%)	1/50 (2%)	1/49 (2%)
MANDIBULAR SALIVARY GLAND				
Metastatic fibrosarcoma	0/48 (0%)	0/50 (0%)	1/49 (2%)	0/49 (0%)
THYMUS				
Malignant lymphoma	0/25 (0%)	0/32 (0%)	1/37 (3%)	1/34 (3%)
Thymoma	0/25 (0%)	0/32 (0%)	1/37 (3%)	0/34 (0%)
MEDIASTINAL LYMPH NODE				
Reticulum cell sarcoma	0/33 (0%)	1/29 (3%)	0/37 (0%)	0/30 (0%)
Malignant lymphoma	0/33 (0%)	0/29 (0%)	1/37 (3%)	2/30 (7%)
SPLEEN				
Malignant lymphoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Reticulum cell sarcoma	2/50 (4%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
STOMACH				
Malignant lymphoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
JEJUNUM				
Leiomyosarcoma	0/50 (0%)	1/48 (2%)	0/49 (0%)	0/49 (0%)
ILEUM				
Reticulum cell sarcoma	0/47 (0%)	0/49 (0%)	0/49 (0%)	1/48 (2%)
COLON				
Reticulum cell sarcoma	0/50 (0%)	0/50 (0%)	0/49 (0%)	1/48 (2%)
URINARY BLADDER				
Transitional cell tumour	0/50 (0%)	0/48 (0%)	0/48 (0%)	1/44 (2%)

Table A4. Incidence of neoplastic findings in female rats with glyphosate administered by diet. Part II. Data extracted from Lankas & Hogan (1981) [17].

Glyphosate /mg kg <sup>-1</sup> day <sup>-1</sup>	0	3	10	30
OVARY				
Granulosa cell tumour	8/49 (16%)	8/50 (16%)	6/48 (13%)	6/45 (13%)
Theca-granulosa cell tumour	0/49 (0%)	0/50 (0%)	0/48 (0%)	1/45 (2%)
UTERUS				
Squamous cell carcinoma	0/50 (0%)	0/50 (0%)	0/49 (0%)	1/49 (2%)
Endometrial sarcoma	0/50 (0%)	0/50 (0%)	0/49 (0%)	1/49 (2%)
Adenoma	0/50 (0%)	0/50 (0%)	2/49 (4%)	1/49 (2%)
THYROID				
C-cell adenoma	5/47 (10%)	3/49 (6%)	6/50 (12%)	3/47 (6%)
C-cell carcinoma	1/47 (2%)	0/49 (0%)	2/50 (4%)	6/47 (12%)
Metastatic fibrosarcoma	0/47 (0%)	0/49 (0%)	1/50 (2%)	0/47 (0%)
PARATHYROID				
Adenoma	0/23 (0%)	0/25 (0%)	0/25 (0%)	1/23 (4%)
ADRENAL				
Reticulum cell sarcoma	1/50 (2%)	1/50 (2%)	1/50 (2%)	3/49 (6%)
Pheochromo-cytoma	1/50 (2%)	2/50 (4%)	2/50 (4%)	2/49 (4%)
Cortical adenoma	5/50 (10%)	10/50 (20%)	6/50 (12%)	4/49 (8%)
Malignant lymphoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/49 (2%)
MAMMARY GLAND (L&R)				
Adenoma (L)	4(47) (8%)	7(46) (15%)	10(48) (20%)	5(44) (11%)
Adenoma (R)	4(47) (8%)	7(46) (15%)	8(48) (16%)	5(44) (11%)
Fibroadenoma (L)	33/47 (66%)	28(46) (61%)	27(48) (56%)	22(44) (50%)
Fibroadenoma (R)	24(47) (48%)	16(46) (35%)	20(48) (41%)	16/44 (36%)
EYE				
Periocular fibrosarcoma	0/49 (0%)	0/48 (0%)	1/50 (2%)	0/47 (0%)
HARDERIAN GLAND				
Malignant lymphoma	0/47 (0%)	0/45 (0%)	0/47 (0%)	1/44 (2%)
Invasive fibrosarcoma	0/47 (0%)	0/45 (0%)	1/47 (2%)	0/44 (0%)
BONE MARROW				
Malignant lymphoma	0/46 (0%)	0/44 (0%)	1/46 (2%)	1/45 (2%)
Reticulum cell sarcoma	1/46 (2%)	0/44 (0%)	1/46 (2%)	3/45 (6%)
SUBCUTANEOUS TISSUE				
Lipoma	0/4 (0%)	0/6 (0%)	0/1 (0%)	2/2 (100%)
Reticulum cell sarcoma	0/4 (0%)	2/6 (33%)	0/1 (0%)	0/2 (0%)
MEDIASTINAL TISSUE				
Reticulum cell sarcoma	0/2 (0%)	1/1 (100%)	0/2 (0%)	0/2 (0%)
MESENTERY				
Reticulum cell sarcoma	0/5 (0%)	0/5 (0%)	0/2 (0%)	2/7 (29%)
MANDIBULAR LYMPH NODE				
Malignant lymphoma	0/2 (0%)	0/3 (0%)	0/6 (0%)	1/6 (17%)
URETER				
Transitional cell carcinoma	0/0 (0%)	0/0 (0%)	1/1 (100%)	1/1 (100%)

Table A5. Incidence of neoplastic findings in male mice with glyphosate administered by diet. Part I. From Knezevich &amp; Hogan, 1983 [18]. BN = Benign, MG = Malignant, MS = Metastatic.

Glyphosate (ppm)	0	Low (1000)	Mid (5000)	High (30000)
BRAIN				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/49 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
HEART				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/47 (0%)	1/49 (2%)	2/49 (4%)	1/50 (2%)
LUNGS				
BN Bronchiolar-alveolar adenoma	5/48 (10%)	9/50 (18%)	9/50 (18%)	9/50 (18%)
MG Bronchiolar-alveolar adeno-carcinoma	4/48 (8%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/48 (2%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma	0/48 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
LIVER				
MG Hepatocellular adenocarcinoma	5/49 (10%)	6/50 (12%)	6/50 (12%)	4/50 (8%)
BN Hepatocellular adenoma	0/49 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
MG Hepatocellular carcinoma	0/49 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
MS Histiocytic sarcoma	0/49 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
MS Liposarcoma	0/49 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49 (2%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
MESENTERIC LYMPH NODES				
MG Histiocytic Sarcoma with leukaemic manifestations	0/40 (0%)	1/50 (2%)	0/46 (0%)	0/49 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	1/40 (2%)	2/50 (4%)	1/46 (2%)	0/49 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/40 (0%)	0/50 (0%)	1/46 (2%)	2/49 (4%)
MG Lymphoblastic lymphosarcoma	0/40 (0%)	1/50 (2%)	0/46 (0%)	0/49 (0%)
MEDIASTINAL LYMPH NODES				
MS Histiocytic sarcoma	0/45 (0%)	1/49 (2%)	0/41 (0%)	0/49 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/45 (2%)	2/49 (4%)	1/41 (2%)	2/49 (4%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	0/45 (0%)	0/49 (0%)	2/41 (5%)	0/49 (0%)

Table A6. Incidence of neoplastic findings in male mice with glyphosate administered by diet. Part II. From Knezevich & Hogan, 1983 [18]. BN = Benign, MG = Malignant, MS = Metastatic.

Glyphosate (ppm)	0	Low (1000)	Mid (5000)	High (30 000)
SPLEEN				
MG Hemangio-endothelioma	0/48 (0%)	0/49 (0%)	1/50 (2%)	0/49 (0%)
MS Histiocytic sarcoma	0/48 (0%)	1/49 (2%)	0/50 (0%)	0/49 (0%)
MS Lymphoblastic lymphosarcoma	1/48 (2%)	2/49 (4%)	2/50 (4%)	0/49 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	0/48 (0%)	2/49 (4%)	0/50 (0%)	1/49 (2%)
PANCREAS				
MS Histiocytic Sarcoma	0/48 (0%)	1/48 (2%)	0/50 (0%)	0/49 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/48 (0%)	0/48 (0%)	1/49 (2%)	0/50 (0%)
KIDNEYS				
BN Renal tubule adenoma	0/49 (0%)	0/49 (0%)	1/50 (2%)	3/50 (6%)
MS Histiocytic sarcoma	0/49 (0%)	1/49 (2%)	0/50 (0%)	0/50 (0%)
MS Composite lymphosarcoma	1/49 (2%)	0/49 (0%)	0/50 (0%)	0/50 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49 (2%)	3/49 (6%)	2/50 (4%)	2/50 (4%)
ADRENAL GLANDS				
BN Cortical adenoma	1/48 (2%)	2/49 (4%)	0/50 (0%)	1/48 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/48 (0%)	1/49 (2%)	0/50 (0%)	0/48 (0%)
BN Lymphoblastic lymphosarcoma with leukaemic manifestations	0/48 (0%)	0/49 (0%)	1/49 (2%)	0/48 (0%)
HARDERGIAN GLAND				
BN Adenoma	1/47 (2%)	0/48 (0%)	0/45 (0%)	0/48 (0%)
MG Liposarcoma	0/47 (0%)	0/48 (0%)	1/45 (2%)	0/48 (0%)
BONE MARROW				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/40 (2%)	2/45 (4%)	1/47 (2%)	1/49 (2%)
LYMPH NODE				
MS Histiocytic sarcoma	0/0 (0%)	1/3 (33%)	0/2 (0%)	0/2 (0%)
MS Composite lymphosarcoma	0/0 (0%)	0/3 (0%)	1/2 (50%)	0/2 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/0 (0%)	1/3 (33%)	1/2 (50%)	0/2 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	0/0 (0%)	0/3 (0%)	0/2 (0%)	1/2 (50%)
TESTES				
BN Interstitial cell tumor	1/49 (2%)	0/48 (0%)	2/50 (4%)	0/50 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/49 (0%)	1/48 (2%)	0/50 (0%)	0/50 (0%)
BN Lymphoblastic lymphosarcoma with leukaemic manifestations	0/49 (0%)	0/48 (0%)	1/50 (2%)	0/50 (0%)



Table A7. Incidence of neoplastic findings in female mice with glyphosate administered by diet. Part I. From Knezevich &amp; Hogan, 1983 [18]. BN = Benign, MG = Malignant, MS = Metastatic.

Glyphosate (ppm)	Controls 0	Low Low (1000)	Mid Mid (5000)	High High (30 000)
BRAIN				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/50 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
HEART				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/49(0%)
LUNGS				
BN Bronchiolar-alveolar adenoma	10/49 (20%)	9/50 (18%)	10/49 (20%)	1/50 (2%)
MG Bronchiolar-alveolar adenocarcinoma	1/49 (2%)	3/50 (6%)	4/49 (8%)	4/50 (8%)
BN Granulosa cell tumour	0/49 (0%)	1/50 (2%)	0/49 (0%)	0/50 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49 (2%)	2/50 (4%)	5/49 (10%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma	0/50 (0%)	0/50 (0%)	0/49 (0%)	1/50 (2%)
LIVER				
MG Hepatocellular adenocarcinoma	1/49 (2%)	2/50 (4%)	1/49 (2%)	0/49 (0%)
BN Hepatocellular adenoma	0/49 (0%)	1/50 (2%)	0/49 (0%)	0/49 (0%)
MS Leiomyosarcoma	0/49 (0%)	1/50 (2%)	0/49 (0%)	0/49 (0%)
MS Granulocytic leukaemia	0/49 (0%)	3/50 (6%)	0/49 (0%)	0/49 (0%)
MG Hemangioendothelioma	0/49 (0%)	0/50 (0%)	2/49 (4%)	0/49 (0%)
MS Composite lymphosarcoma	2/49 (4%)	1/50 (2%)	0/49 (0%)	4/49 (8%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49 (2%)	4/50 (8%)	4/49 (8%)	1/49 (2%)
MS Lymphoblastic lymphosarcoma	0/49 (0%)	0/50 (0%)	0/49 (0%)	2/49 (4%)
MESENTERIC LYMPH NODES				
MS Leiomyosarcoma	0/49 (0%)	1/49 (2%)	0/48 (0%)	0/48 (0%)
MS Granulocytic leukaemia	0/49 (0%)	1/49 (2%)	0/48 (0%)	0/48 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	0/49 (0%)	3/49 (6%)	1/48 (2%)	1/48 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49 (2%)	1/49 (2%)	3/48 (6%)	0/48 (0%)
MS Composite lymphosarcoma	1/49 (2%)	1/49 (2%)	1/48 (2%)	3/48 (6%)
MG Lymphoblastic lymphosarcoma	0/49 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
MS Lymphoblastic lymphosarcoma	0/49 (0%)	0/49 (0%)	0/49 (0%)	1/49 (2%)
MS Haemangioendothelioma	0/49 (0%)	0/49 (0%)	0/49 (0%)	1/49 (2%)

Table A8. Incidence of neoplastic findings in female mice with glyphosate administered by diet. Part II. From Knezevich & Hogan, 1983 [18]. BN = Benign, MG = Malignant, MS = Metastatic.

Glyphosate (ppm)	Controls 0	Low Low (1000)	Mid Mid (5000)	High High (30 000)
MEDIASTINAL LYMPH NODES				
MS Leiomyosarcoma	0/42 (0%)	1/48 (2%)	0/39 (0%)	0/47 (0%)
MS Granulocytic leukaemia	0/42 (0%)	1/48 (2%)	0/39 (0%)	0/47 (0%)
MS Liposarcoma	1/42 (2%)	0/48 (0%)	0/39 (0%)	0/47 (0%)
MS Composite lymphosarcoma	1/42 (2%)	1/48 (2%)	0/39 (0%)	2/47 (4%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/42 (0%)	1/48 (2%)	3/39 (8%)	0/47 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	1/42 (2%)	1/48 (2%)	2/39 (5%)	0/47 (0%)
MS Lymphoblastic lymphosarcoma	0/42 (0%)	1/48 (2%)	0/39 (0%)	1/47 (2%)
SALIVARY GLAND				
MS Leiomyosarcoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/47 (0%)
SPLEEN				
MG Hemangio-endothelioma	1/50 (2%)	0/48 (0%)	2/49 (4%)	1/49 (2%)
MG Granulocytic leukemia	0/50 (0%)	3/48 (6%)	0/49 (0%)	0/49 (0%)
MS Hemangio-endothelioma	0/50 (0%)	0/48 (0%)	0/49 (0%)	1/49 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/50 (2%)	2/48 (4%)	2/49 (4%)	0/49 (0%)
MG Lymphoblastic lymphosarcoma with leukaemic manifestations	0/50 (0%)	0/48 (0%)	2/49 (4%)	0/49 (0%)
MG Composite lymphosarcoma	1/50 (2%)	1/48 (2%)	1/49 (2%)	5/49 (10%)
MS Lymphoblastic lymphosarcoma	0/50 (0%)	0/48 (0%)	0/49 (0%)	1/49 (2%)
STOMACH				
MG Leiomyosarcoma	0/48 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
MG Gastric adenocarcinoma	0/48 (0%)	0/49 (0%)	1/50 (2%)	0/50 (0%)
PANCREAS				
MS Granulocytic leukaemia	0/47 (0%)	1/47 (2%)	0/49 (0%)	0/50 (0%)
MS Composite lymphosarcoma	2/47 (4%)	1/47 (2%)	0/49 (0%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/47 (2%)	1/47 (2%)	1/49 (2%)	0/50 (0%)
KIDNEYS				
MS Leiomyosarcoma	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
MS Granulocytic leukaemia	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
MS Composite lymphosarcoma	2/50 (4%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/50 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
MS Lymphoblastic lymphosarcoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)

Table A9. Incidence of neoplastic findings in female mice with glyphosate administered by diet. Part III. From Knezevich &amp; Hogan, 1983 [18]. BN = Benign, MG = Malignant, MS = Metastatic.

Glyphosate (ppm)	Controls 0	Low Low (1000)	Mid Mid (5000)	High High (30 000)
URINARY BLADDER				
MS Granulocytic leukaemia	0/47 (0%)	1/43 (2%)	0/49 (0%)	0/48 (0%)
MS Composite lymphosarcoma	1/47 (2%)	1/43 (2%)	0/49 (0%)	0/48 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	1/47 (2%)	2/43 (4%)	2/49 (4%)	0/48 (0%)
OVARIES				
MG Teratoma	0/47 (0%)	1/47 (2%)	0/50 (0%)	0/47 (0%)
MG Granulosa cell tumour	0/47 (0%)	1/47 (2%)	0/50 (0%)	0/47 (0%)
MS Leiomyosarcoma	0/47 (0%)	1/47 (2%)	0/50 (0%)	0/47 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/47 (0%)	1/47 (2%)	0/50 (0%)	0/47 (0%)
MS/BN Lymphoblastic lymphosarcoma with leukaemic manifestations	1/47 (2%)	0/47 (0%)	2/50 (4%)	0/47 (0%)
UTERUS				
MS Leiomyoma	2/49 (4%)	1/48 (2%)	1/49 (2%)	1/50 (2%)
MG Leiomyosarcoma	2/49 (4%)	3/48 (6%)	2/49 (4%)	3/50 (6%)
MG Endometrial stromal cell carcinoma	0/49 (0%)	1/48 (2%)	0/49 (0%)	0/50 (0%)
MS Haemangioma	0/49 (0%)	1/48 (2%)	0/49 (0%)	0/50 (0%)
MG Haemangio-endothelioma	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/50 (0%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/49 (0%)	3/48 (6%)	1/49 (2%)	0/50 (0%)
CERVIX				
MG Leiomyosarcoma	0/0 (0%)	2/2 (100%)	0/0 (0%)	0/1 (0%)
THYROID				
MS Follicular adenoma	0/43 (0%)	0/37 (0%)	1/49 (2%)	0/48 (0%)
SKIN				
MG Fibrosarcoma	0/45 (0%)	1/45 (2%)	1/49 (2%)	0/48 (0%)
MAMMARY				
MG Ductal adenocarcinoma	2/38 (5%)	4/36 (11%)	2/40 (5%)	1/38 (3%)
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/38 (0%)	0/36 (0%)	1/40 (3%)	0/38 (0%)
BONE MARROW				
MS Lymphoblastic lymphosarcoma with leukaemic manifestations	0/46 (0%)	1/49 (2%)	3/47 (6%)	1/49 (2%)
MS Lymphoblastic lymphosarcoma	0/46 (0%)	0/49 (0%)	0/47 (0%)	2/49 (4%)
MS Composite lymphosarcoma	0/46 (0%)	0/49 (0%)	0/47 (0%)	1/49 (2%)

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