

Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence

M Antoniou¹, MEM Habib², CV Howard³, RC Jennings⁴, C Leifert⁵, RO Nodari⁶, CJ Robinson^{7*} and J Fagan^{8*}

¹Head, Gene Expression and Therapy Group, Department of Medical and Molecular Genetics, King's College London School of Medicine, UK

²Professor of entomology, former director, Institute of Biology, UNICAMP, and former provost of extension and community affairs, UNICAMP, São Paulo, Brazil

³Professor, Centre for Molecular Biosciences, University of Ulster, Northern Ireland

⁴Affiliated research scholar, Department of History and Philosophy of Science, University of Cambridge, UK

⁵Research development professor for ecological agriculture at the University of Newcastle, UK. Interests: director and trustee of the Stockbridge Technology Centre Ltd (STC), UK

⁶Professor, Center for Agricultural Sciences (department of plant science), Federal University of Santa Catarina, Brazil

⁷Research director, Earth Open Source, London, UK. Interests: editor, GM Watch, UK

⁸Director, Earth Open Source. Interests: employed at a GMO testing and certification company

Abstract

The publication of a study in 2010, showing that a glyphosate herbicide formulation and glyphosate alone caused malformations in the embryos of *Xenopus laevis* and chickens through disruption of the retinoic acid signalling pathway, caused scientific and regulatory controversy. Debate centred on the effects of the production and consumption of genetically modified Roundup Ready[®] soy, which is engineered to tolerate applications of glyphosate herbicide. The study, along with others indicating teratogenic and reproductive effects from glyphosate herbicide exposure, was rebutted by the German Federal Office for Consumer Protection and Food Safety, BVL, as well as in industry-sponsored papers. These rebuttals relied partly on unpublished industry-sponsored studies commissioned for regulatory purposes, which, it was claimed, showed that glyphosate is not a teratogen or reproductive toxin.

However, examination of the German authorities' draft assessment report on the industry studies, which underlies glyphosate's EU authorisation, revealed further evidence of glyphosate's teratogenicity. Many of the malformations found were of the type defined in the scientific literature as associated with retinoic acid teratogenesis. Nevertheless, the German and EU authorities minimized these findings in their assessment and set a potentially unsafe acceptable daily intake (ADI) level for glyphosate. This paper reviews the evidence on the teratogenicity and reproductive toxicity of glyphosate herbicides and concludes that a new and transparent risk assessment needs to be conducted. The new risk assessment must take into account all the data on the toxicity of glyphosate and its commercial formulations, including data generated by independent scientists and published in the peer-reviewed scientific literature, as well as the industry-sponsored studies.

Keywords: Glyphosate; Roundup; Teratogenicity; Teratogenic effects; Malformations; Risk assessment; Reproductive toxicity

Introduction

An investigation (Paganelli et al.) of the toxicity of a commercial Roundup[®] herbicide formulation and its active ingredient glyphosate found that these substances caused severe malformations in embryos of the South African clawed frog *Xenopus laevis* and chickens. In frogs, dilutions of 1/5000 of the formulation (equivalent to 430 μ M of glyphosate) were sufficient to induce malformations, including shortening of the anterior–posterior axis, microcephaly, micropthalmia, cyclopia, and craniofacial malformations at tadpole stages. Embryos injected with pure glyphosate showed similar phenotypes, suggesting that glyphosate itself, rather than a surfactant or other adjuvant present in the formulation, was responsible for these developmental abnormalities. Roundup[®] produced similar effects in chicken embryos, which showed a loss of rhombomere domains, reduction of the optic vesicles, and microcephaly.

Through the use of reporter gene assays and phenotypic rescue via administration of an antagonist, the authors confirmed that the mechanism by which glyphosate and Roundup caused the observed teratogenic effects in *Xenopus* embryos was via disruption of the retinoic acid signalling pathway. This resulted in dysregulation of the *shh*, *slug* and *otx2* regulatory genes, which are crucial to the development of the central nervous system [1]. The study, while not a classical toxicological study, is relevant to human risk assessment because the retinoic acid signalling pathway is a central signalling pathway in embryonic development that operates in virtually all vertebrates, whether amphibians, birds, or mammals.

Other Studies Showing Malformations from Glyphosate and Roundup Exposure

Paganelli et al.'s study was one among several to find malformations from glyphosate and Roundup exposure. Jayawardena et al. (2010) found nearly 60% malformations in tadpoles of the tree frog *Polypedates cruciger* treated with an environmentally relevant concentration of 1 ppm Roundup. Effects included kyphosis, scoliosis, and edema [2]. Relyea (2012) found that environmentally relevant concentrations of Roundup induced relatively deeper tails similar to the adaptive changes caused by the presence of a predator in the tadpoles of the wood frog (*Rana sylvatica* or *Lithobates sylvaticus*) and leopard frog (*R. pipiens* or *L. pipiens*) [3]. A study on tadpoles of *Scinax nasicus* (Lajmanovich et al., 2005) found that exposure to glyphosate herbicide caused craniofacial and mouth deformities, eye abnormalities and bent, curved tails, as well as mortality. Malformations and mortality increased with dose and time of exposure. A 2-day exposure to 3.07 mg/l glyphosate

***Corresponding authors:** Claire Robinson, Research director, Earth Open Source, London, UK, E-mail: claire.robinson@earthopensource.org

John Fagan, Director, Earth Open Source, E-mail: jfagan64@gmail.com

Received June 01, 2012; Accepted June 21, 2012; Published June 23, 2012

Citation: Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, et al. (2012) Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. J Environ Anal Toxicol S4:006. doi:[10.4172/2161-0525.S4-006](https://doi.org/10.4172/2161-0525.S4-006)

Copyright: © 2012 Antoniou M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

herbicide caused only 10% mortality but caused malformations in 55% of the test animals [4].

Malformations have also been found in mammals treated with glyphosate herbicides. A toxicological study by Dallegrove et al. (2003) found that the offspring of pregnant rats dosed with 500, 750 and 1000mg/kg Roundup on days 6–15 after fertilisation had increased skeletal abnormalities, including at doses that were not maternally toxic. Malformations consisted of the absence of bones or parts of bones, shortened and bent bones, asymmetry, fusions, and clefts. The percentage of altered fetuses increased with dose. The authors concluded that the formulated product was more toxic than the technical glyphosate evaluated by the World Health Organisation [5] and tested in the industry-sponsored teratogenicity studies described in Germany's 1998 draft assessment report on glyphosate.

Scientific and Political Controversy in Europe

In Europe, the publication in 2010 of the study by Paganelli et al. [1] coincided with rising concern over the 40 million tons of soy that are imported each year, mostly to feed livestock. Much of this soy is the genetically modified (GM) Roundup Ready® variety [6], which is engineered to tolerate applications of glyphosate herbicide. Scientific and political debate had continued for many years over the public health, environmental, and socioeconomic consequences of GM soy cultivation in producer countries [7]. More recently, concerns expanded to include potential risks to animal and human health posed by glyphosate residues in the animal feed and human food chain [8,9]. Residues of up to 17 mg/kg of glyphosate have been found in harvested soybean crops [10].

As the existing EU approval of glyphosate dated from 2002 [11] and was valid for ten years, a new review was due in 2012. In response to a Parliamentary question, John Dalli, EU Commissioner for health and consumer policy, stated that the date might be brought forward if the new evidence justified it [12].

German Authorities and Industry Respond

Commissioner Dalli gave the task of assessing Paganelli et al.'s findings to the German regulatory authorities. As the "rapporteur" member state for glyphosate, Germany was responsible for liaising between industry, the European Commission, and the member states in the authorisation process. In October 2010 Germany's Federal Office for Consumer Protection and Food Safety, BVL, issued an anonymous rebuttal of the study, which stated:

There is a huge and reliable database for developmental toxicity of glyphosate and no evidence of teratogenicity has been obtained. In particular, studies in rats and rabbits failed to reveal craniofacial malformations as ... would be expected if a substance affects mainly the neural crest [13].

While the identity of the "huge and reliable database" is not defined by BVL, the studies on rats and rabbits to which BVL referred were those commissioned by manufacturers of glyphosate herbicides and summarised in Germany's 1998 draft assessment report on glyphosate. Germany's summary of, and commentary on, these largely unpublished, non-peer-reviewed industry-sponsored studies in the draft assessment report form the basis of the EU's current authorisation of glyphosate, which dates from 2002 [11].

BVL concluded that Paganelli et al.'s findings "do not put the current risk assessment for glyphosate and glyphosate-based PPP

[plant protection products – pesticides] into question with regard to human health" [13].

Based on BVL's assessment, Commissioner Dalli said there was no need to restrict or ban glyphosate [14]. The Commission did not bring the glyphosate review forward, or even keep to the expected date of 2012. Instead, in November 2010 it issued a Directive delaying the review of glyphosate until 2015 [15]. In response to a query from Friends of the Earth Germany as to the reason for the delay, BVL replied that the Commission and the European Food Safety Authority (EFSA) had too heavy a workload to review glyphosate and had not finalized the rules for renewing the approval of certain pesticides, including glyphosate (personal communication from BVL to Friends of the Earth Germany).

BVL's response to Paganelli et al. was followed in 2011 by a similar response from industry. Employees of Monsanto, Dow, and Syngenta, all manufacturers of glyphosate herbicides, published a letter in the same journal that published Paganelli et al.'s original paper [16]. The Monsanto/Dow/Syngenta letter, published alongside a reply from Andres Carrasco, lead author of the Paganelli et al. Paper [17], stated: "Glyphosate does not cause adverse reproductive effects in adult animals or birth defects in offspring of these adults exposed to glyphosate, even at very high doses" [16].

Claims of No Teratogenicity Assessed

In order to test BVL's claim of the absence of teratogenicity in the industry studies on glyphosate, we obtained from the German authorities the draft assessment report on glyphosate that they compiled in 1998. The industry toxicological data summarised in the draft assessment report are not publicly available and are claimed by Monsanto to be commercially confidential, though Pesticide Action Network Europe is pursuing disclosure through the courts (personal communication).

Examination of the draft assessment report revealed that the industry toxicological studies on rabbits and rats that BVL said showed "no evidence of teratogenicity" did, in fact, report malformations from glyphosate exposure [12].

In the draft assessment report, the German authorities concluded, based on the industry studies, "Glyphosate does not cause teratogenicity", but added that higher doses of glyphosate caused "reduced ossification and a higher incidence of skeletal and/or visceral anomalies" in rats and rabbit fetuses, as well as a reduced number of viable fetuses [18]. The latter is consistent with increased incidence of malformations. The German authorities do not define what they mean by "higher" doses, but the industry-sponsored teratogenicity studies typically use three doses: low, mid, and high dose. For details of the dose levels used, see Table 1.

However, in the industry-sponsored studies, malformations were found not only at high, maternally toxic doses, as the Commission's health and consumer affairs division, DG SANCO, stated in its 2002 review report on glyphosate [11], but also at lower doses. In some cases, effects at lower doses were statistically significant [12], though statistical significance at lower doses is difficult to obtain in standardised industry studies performed for regulatory purposes, which use small numbers of animals per group.

Table 1, below, shows the range of malformations found in the industry teratogenicity studies on glyphosate, as summarised by the German authorities in the draft assessment report. The studies were conducted as long ago as the 1980s and 1990s. Many of the

malformations found are consistent with descriptions of retinoic acid-induced teratogenesis in the literature. For example, the increased heart malformations and abnormalities noted in the draft assessment report are cited as characteristic of retinoic acid-induced teratogenicity by Lammer et al. [19], Kessel [20], and Huang et al. [21]. The supernumerary, distorted, and rudimentary ribs noted in the draft assessment report are consistent with Kessel's (1992) citation of the generation of supernumerary ribs and rib malformations as characteristic of retinoic acid-induced teratogenicity [20]. Absent postcaval lobe of the lungs, as mentioned in the draft assessment report, is consistent with the lung dysmorphogenesis caused by retinoic acid administration or deprivation as cited in Malpel [22], Wilson et al. [23], Shenefelt [24] and Dickman et al. [25]. The reduced ossification of cranial centres and sacro-caudal vertebral arches, as well as the undefined skeletal malformations, cited in the draft assessment report are consistent with the cranial and skeletal malformations cited by Lammer et al. [19], Kessel [20] and Huang et al. [21] as characteristic of retinoic acid-induced teratogenicity.

General Observations on the Draft Assessment Report

Maternal toxicity

Throughout the draft assessment report, German regulators dismissed findings of malformations in industry studies by claiming that the effects were due to maternal toxicity. In its 2002 report that forms the basis of the EU authorisation of glyphosate, the EU Commission's health and consumer division, DG SANCO, followed the German regulators' lead, discounting the developmental abnormalities on the grounds that they were confined to "maternally toxic doses" [11], though how this conclusion was reached is unclear.

The general reasoning behind this conclusion is that poisoning of the mother with any substance (including commonly ingested substances like salt and caffeine) could affect the development of the foetus and therefore such malformations are not a direct and specific effect of the substance on the foetus. Germany and DG SANCO argued that the studies report maternal toxicity and therefore the foetal abnormalities were due to maternal toxicity. However, the studies failed to differentiate between maternal toxicity and compound-specific teratogenicity. At the high doses used, both could be taking place.

It is unfortunate that the standardised industry studies performed for regulatory purposes use only a small number of animals per group. Given this restriction, relatively high doses of the test compound are used and maternal toxicity effects are common. There is a high risk that this type of study design can miss compound-specific effects that occur at low- and medium- frequency.

An equally valid conclusion that could be drawn from the industry studies is that maternal toxicity could be obscuring a compound-specific teratogenic effect and may not be the only cause of the observed malformations. This argues that another study should have been conducted employing larger groups of animals and lower, more realistic doses administered over a longer, preferably lifelong, period before the possibility of compound-specific teratogenic effects could be eliminated and before glyphosate could be deemed free from teratogenic effects.

Paumgarten (2010) supports this conclusion, stating that in cases of maternal toxicity, it is not possible to know whether an effect on the embryo is due to non-specific maternal poisoning or to a direct action of the chemical at doses that also adversely affect the mother. In the latter case, the chemical would be a developmental toxin [26].

Dallegrave et al. (2007) demonstrated that this issue is relevant to glyphosate formulations. The authors examined the effects of Roundup on reproduction in male and female offspring of rats treated during pregnancy and lactation with doses of Roundup that were too low to induce maternal toxicity. They found that Roundup at these doses induced adverse reproductive effects in male offspring, showing that this herbicide formulation is a reproductive toxin at non-maternally toxic doses [27].

This argues for the need to redesign regulatory tests to use larger groups of animals and more realistic doses over longer exposure periods, preferably beginning exposures prenatally.

Nonlinear Dose-Response

Throughout the draft assessment report, German regulators assumed that only effects that follow a linear dose-response relationship are valid. But this assumption is not supported by current knowledge. Dose-response relationships can be complex and nonlinear, especially where the endocrine system is involved. A large body of evidence indicates that for some compounds, toxic effects are found at low doses but not at higher doses, and that different toxic effects can be found at different doses [28-31].

Roundup and glyphosate have been found to be endocrine disruptors [27,32], and therefore, nonlinear dose-responses may apply for some endpoints. Indeed, a neurotoxicity study found not only that Roundup was more toxic than glyphosate and produced effects at concentrations as low as 10 ppb or 0.01 micrograms/L (equivalent to a glyphosate concentration of 0.5 nM), but also found "unusual" dose-response relationships with both substances, suggesting that low-dose effects may not be predictable from effects at high doses [33].

We conclude that it is not in accord with current scientific understanding to dismiss findings of increased developmental malformations on the grounds of a nonlinear dose-response relationship. However, given that that current practice by regulatory agencies assumes that the dose-response relationship should be linear, we indicate, where relevant in Table 1, where such a relationship is observed.

Historical control data

The German regulators repeatedly discounted findings of malformations in industry studies by referring to historical control data instead of the concurrent controls measured in the studies themselves.

However, the use of this historical control data set is questionable from two perspectives. First, the draft assessment report fails to disclose the historical control data used. It does not provide the individual data points or statistical measures of the variability within the dataset. Second, the draft assessment report fails to present evidence that demonstrates the validity of this historical control dataset.

The use of historical, instead of concurrent, controls is contrary to rigorous scientific methodology, which is designed to tightly control for variables. It artificially introduces variables into the dataset, potentially masking any effect caused by the substance being tested. Potential variables include:

- strain of animal, involving a different genetic background and sensitivity.
- substance tested, introduced by different manufacturing processes and storage conditions.

Study author and date	Submitter company	Experimental animal/exposure route	Doses used mg/kg bw/d	Effects found	Dose-related effects/Statistical significance
Suresh, 1993	Feinchemie	Rabbits/gavage	0, 20, 100, 500	Dilated heart	Linear dose-response relationship. Significantly elevated at all doses, including low dose
				Unspecified "major visceral malformations"	Linear dose-response relationship. Increased in all treatment groups, significantly increased at highest dose
				Extra 13 th rib	Linear dose-response relationship. Statistically significant increase at highest dose
Brooker et al., 1991	Monsanto/Cheminova	Rabbits/gavage	0, 50, 150, 450	Heart malformations (only type specified is interventricular septal defect)	Effect found at highest dose. No information provided by Germany on statistical significance
				Embryonic deaths	Significant at all doses, though no clear dose/response relationship
Bhide and Patil, 1989	Barclay/Luxan	Rabbits/route unstated	0, 125, 250, 500	Heart malformation (ventricular septal defect)	Linear dose-response relationship. No statistical analysis provided by authors. Increased heart malformations found in all treatment groups
				Lungs: postcaval lobe absent	Linear dose-response relationship. No statistical analysis provided by authors. Dose-dependent increases found in all treatment groups
				Kidneys absent	Linear dose-response relationship. No statistical analysis provided by authors. Dose-dependent increases found in all treatment groups
				Rudimentary 14 th rib, unilateral	No statistical analysis provided by authors. Dose-dependent increases found in mid- and high-dose groups
				Number of viable foetuses per litter decreased and number of non-viable implants increased	Linear dose-response relationship in case of non-viable implants. No statistical analysis provided by authors. Effects found at high dose level.
Tasker et al., 1980	Monsanto/Cheminova	Rabbits/gavage	0, 75, 175, 350	Increased number of deaths in dams	Linear dose-response relationship. 1, 2, and 10 deaths in low, mid- and high-dose treatment groups respectively (no. of rabbits per group: 16 or 17). 75 mg/kg stated by Germany to be NOAEL
Anon., 1981	Alkaloida	Rats and rabbits/oral feeding	0, 10.5, 50.7, 255.3	Increased number of foetal deaths	Effect seen at two upper dose levels
Zhu et al., 1984	Barclay	Mice/gavage	80, 420, 1050	Germany comments that there is "No evidence of dose-related toxic effects" and "no ... structural malformations" but that description of experiment was "poor"	Data not provided by Germany
Brooker et al., 1991	Monsanto/Cheminova	Rats/gavage	0, 300, 1000, 3500	Distortions affecting thoracic ribs	Dose-dependent increases found in mid- and high-dose groups. Statistically significant at high dose
				Reduced ossification of one or more cranial centres	Dose-dependent increases found in mid- and high-dose groups.
				Reduced ossification of sacro-caudal vertebral arches	Dose-dependent increases found in mid- and high-dose groups
				Unossified sternebrae	Increases found in all treated groups, statistically significant at high dose
				Skeletal variations	Dose-dependent increases found in all treated groups, statistically significant in mid-dose and high-dose groups.
Tasker and Rodwell, 1980	Monsanto/Cheminova	Rats/gavage	0, 300, 1000, 3500	Unossified sternebrae	Increase found at highest dose level
				Unspecified malformations	Increase at highest dose level
				No. of viable foetuses per litter and mean foetal weight decreased	Effects found at highest dose level
				Early resorption of embryos	Data not provided by Germany

Suresh, 1991	Feinchemie	Rats/gavage	0, 1000	Increase in delayed ossification (caudal vertebral arch, forelimb proximal and hindlimb distal phalanges) found in treatment group, but increase in delayed ossification of skull found in control group	Conflicting data led Germany to conclude that the NOAEL for developmental toxicity was 1000 mg/kg
Bhide, 1986	Barclay/Luxan	Rats/gavage	0, 100, 500	No effects found but Germany commented that "serious reporting deficiencies" and lack of statistical analysis led it to consider the study as supplementary information only	In spite of lack of reliable data, Germany derived a NOAEL for developmental toxicity of 500 mg/kg
Anon., 1981	Alkaloida	Rats and rabbits/oral feeding	22, 103, 544	Germany commented that description of study is so "poor" that it only considered the study as supplementary information. There were "no malformations recorded" and effects on foetuses were "not observed" but it is unclear from Germany's summary whether this was due to poor reporting by the study's authors or if there was an actual absence of effects	In spite of lack of reliable data, Germany derived a NOAEL for developmental toxicity of 544 mg/kg

Source for all studies: Rapporteur member state Germany (1998) Monograph on glyphosate, German Federal Agency for Consumer Protection and Food Safety (BVL). Vol 3-1 Glyphosat 05: pp. 9–20

Table 1: Malformations, embryonic deaths, and maternal deaths in industry-sponsored teratogenicity studies on glyphosate.

- diet for the experimental animals, which can vary in composition and contaminants.
- pathogens in the environment.
- year and laboratory in which the experiments were performed, for unknown reasons [34].

In order to demonstrate that the use of these historical control data for glyphosate is valid, German regulators must disclose the datasets used and demonstrate the relevance of each data point included in the dataset. In the absence of such documentation, we consider the conclusions of the draft assessment report to be questionable.

Several reviews state that concurrent control groups are the most valid controls and warn against the biasing effect of conducting comparisons with historical control data [34-36]. Cuffe (2011) stated that using such data can lead to Type II errors [36]. In the case of glyphosate, this would be a false negative, in which a finding of toxicity was overlooked.

In rare instances, the use of historical control data is acceptable, such as where effects observed are borderline, showing only a marginal increase over concurrent controls, or in the case of rare tumours, where data other than historical data is unavailable. Nevertheless, extreme care must be taken in selecting the data points included in the historical control dataset. Specifically, all sources of variability in the historical control data must be identified and controlled for [34]. There is no evidence in the draft assessment report that the German regulators did this in the case of glyphosate.

Analysis of industry-sponsored studies from the draft assessment report

Below we analyse selected industry-sponsored teratogenicity studies from the German regulators' draft assessment report (see Table 1 for a summary of all the industry teratogenicity studies cited in the draft assessment report). For each study analysed, we present: (a) a condensed version of the German regulators' summary of the findings; (b) the comments of the UK's Pesticides Safety Directorate (PSD), where relevant; and (c) our comments.

Increased skeletal, visceral, and heart malformations

With regard to a study in rabbits by Suresh:

- The German regulators stated that this study found that the

total number of foetuses with major visceral anomalies was high in all treated groups, including the low dose level of 20 mg/kg glyphosate, and was significantly increased at the highest dose level of 500 mg/kg. The percentage of foetuses with dilated heart was significantly elevated at all dose levels. Skeletal variations, anomalies and malformations were found but there was no clear dose-response pattern. There was a dose-related increase in the occurrence of an extra 13th rib in all glyphosate-treated groups; in the high dose group this was statistically significant.

The German regulators dismissed the findings on the grounds that the actual number of foetuses with dilated heart was small, that there was no increase in foetuses with heart dilation in the mid-dose over the low-dose group, that almost no other soft organ malformations occurred, and that the supposed consequences of this heart malformation were "equivocal". They concluded that the low dose of 20 mg/kg bw/d and even the mid dose of 100 mg/kg bw/d were NOAELs (No Observable Adverse Effect Levels) [37].

- The UK's Pesticides Safety Directorate (PSD) commented, "The increased incidences of abnormalities ... are of concern, particularly the heart effects which are also reported in other rabbit studies with glyphosate... The interpretation of this finding must rely on comparison with historical control data" [38].
- In fact, no NOAEL was found in this study, as a statistically significant increase in the dilated heart malformation was found even at the lowest dose of 20 mg/kg. Therefore, the German regulators should have asked for further tests at lower doses to establish a true NOAEL. Their comment that the number of foetuses with abnormalities was small merely identifies a shortcoming of the standardised industry studies performed for regulatory purposes. Larger numbers of animals are preferable. If the number of animals used is small, any effect will only be seen in a few animals and statistical significance will be difficult to obtain. This is especially true at lower doses, where observable effects will be smaller and/or less frequent.

The German regulators' dismissal of the heart malformations on the grounds that no other soft organ malformations were found is invalid, as toxic agents can have organ-specific effects. Their argument that the heart dilation malformation had "equivocal" consequences and could therefore be dismissed is scientifically and clinically indefensible.

Increased heart malformations and embryonic deaths

With regard to a study in rabbits by Brooker et al.:

- a. The German regulators stated that this study on the effects of glyphosate on pregnancy in rabbits found a significant increase in embryonic deaths in all treatment groups. However, they argued that comparison with historical control data showed that the incidence in the concurrent control group was untypically low and that therefore the increase was not significant. In addition, they questioned its biological significance, arguing that a clear dose-response relationship was not shown. The German regulators did, however, state that an increase in late embryonic deaths at the highest dose level had been reported in another study. They noted the increased incidence of heart malformations in the high dose group, but stated that this was within the range of historical control data. They added that anomalies of the heart were found in other teratogenicity studies with glyphosate in rabbits, but concluded that a possible effect on the occurrence of visceral anomalies was “equivocal” [39].
- b. The UK PSD commented: “The increased levels of embryonic death/post-implantational loss at all dose levels are of concern, as are the reports of heart defects... a more robust argument should be presented before these findings can be dismissed” [38].
- c. Again, the German regulators used historical control data and an inappropriate model for toxicity dose-response to dismiss heart malformations. We believe that this conclusion was not justified and that the increase in late embryonic deaths required investigation because malformed foetuses are often spontaneously aborted or are born dead. The relevance of this observation to humans is suggested by a study of farm families in Ontario, Canada, which found a higher than normal rate of miscarriages and pre-term deliveries associated with glyphosate herbicide exposure [40].

Decrease in viable foetuses, increase in malformations

With regard to a study in rabbits by Bhide and Patil:

- a. The German regulators stated that this study found a decreased number of viable foetuses per litter and increased embryonic deaths. The number of visceral and skeletal malformations was increased in the high-dose group [41].
- b. The UK PSD commented: “Another study with equivocal evidence of heart defects” [42].
- c. Dose-dependent increases in lung and kidney malformations were found across all treatment groups. Increased frequency of heart malformations was found in all treatment groups. Increased skeletal (rudimentary 14th rib) malformations, typical of retinoic acid embryopathy, were found in the mid-dose and high-dose groups.

German regulators incorrectly stated that the teratogenic NOAEL was the mid dose of 250 mg/kg bw/d. In fact, the data showed increases in most defects even at the low dose of 125 mg/kg bw/d. The authors of this study did not provide an analysis of statistical significance and groups of only 15 animals were used, making it difficult to achieve statistical power at lower doses. The data presented in this study suggest that it would be more appropriate to declare the mid dose, possibly even

the low dose of 125 mg/kg, as the LOAEL (lowest observed adverse effect level) and to state that no NOAEL was found.

Increased foetal deaths

With regard to a study in rats and rabbits by Anon:

- a. The German regulators stated that this oral feeding study was poorly recorded and was only considered as supplementary information. No malformations were recorded, but there were more foetal deaths at the two upper dose levels (50.7 and 255.3 mg/kg bw/d). They stated that it was difficult to understand why an increase in foetal deaths would occur at doses far below those at which foetal effects were found in gavage studies and concluded that it was “doubtful” whether this effect was treatment-related [43].
- b. The UK PSD commented, “Though this study is questioned [by German regulators] for showing evidence of foetotoxicity at lower doses than other studies, the study by Brooker (see above) may also indicate foetotoxicity at 50 mg/kg bw/d” [42].
- c. The German regulators’ assumption that low-dose findings were non-treatment-related because oral feeding resulted in different effects than gavage is not defensible. As was pointed out by the UK’s PSD, another study supported this study’s findings [42]. There is no explanation in the draft assessment report as to whether, or how, this disagreement was resolved, and thus the issue remains open for discussion.

Increased unossified sternebrae

With regard to a study in rats by Tasker and Rodwell:

- a. The German regulators stated that this study found a higher number of foetuses with malformations at the highest dose level (3500 mg/kg bw/d), but considered that this was within the range of historical control data and not treatment-related. Specifically, there were more foetuses with unossified sternebrae in the high-dose group. While they accepted that this effect was treatment-related, they concluded that it was “rather a developmental variation than a malformation” [44].
- b. The German regulators again used historical control data to dismiss evidence of teratogenicity. Given the findings of malformations in other studies, this is not justified. To define unossified sternebrae as a “developmental variation” rather than a malformation is scientifically unjustifiable. Unossified sternebrae in the rat are clearly defined as a skeletal deformity in *The Handbook of Pesticide Toxicology* [45].

Increased skeletal malformations

With regard to a study in rats by Brooker et al.:

- a. The German regulators stated that this gavage study in rats found increased incidence of reduced ossification and increased skeletal malformations at the mid and high doses but added that the results were within the range of historical control data. They stated that maternal toxicity was a confounding factor and described the significance of the malformations as “equivocal” [46].
- b. Again, the German regulators used historical control data and maternal toxicity to minimize the significance of malformations. However, these malformations are consistent with the findings

of Paganelli et al. and are associated with disturbances in the retinoic acid signalling pathway [1].

PSD's conclusion

The UK PSD's overall conclusion on the industry-sponsored rabbit teratogenicity studies was: "Taken in isolation, none of the findings ... would be clearly of concern. However, overall there is an indication of a pattern" (our emphasis). The PSD asked the German regulatory authorities to make available the historical control data against which they compared the findings of these studies [42], but we have been unable to locate any published statement indicating whether the PSD saw this data or, if it did, how it responded.

Following the deliberations of the German regulators and the UK's PSD, the issue of glyphosate-mediated teratogenicity was considered by the EU Commission's ECCO scientific review panel. The ECCO panel noted "the incidence of heart malformations", but dismissed them on the grounds that they were "within the range of the historical control data" [47]. Again, it is unclear from the panel's statement whether it saw the historical control data and, if so, whether it systematically assessed the validity of that data set. Subsequently, in 2002, the EU Commission authorised glyphosate.

Misleading "Safe" Level Set For Glyphosate?

The central purpose of a pesticide risk assessment is to establish an Acceptable Daily Intake (ADI), a level of exposure deemed safe for humans over a long period. In the case of glyphosate, the ADI was calculated from the dataset provided by industry-sponsored studies, some of which are discussed above. The level that should be used to set the ADI is the highest dose at which no adverse effect is observed (NOAEL), which is also lower than the lowest dose at which adverse effects are observed (LOAEL). This level should be selected from "the most appropriate study in the most sensitive species", as the German regulators note [48].

The German regulators set the ADI for glyphosate at 0.3 mg/kg bw/d [49]. This ADI was accepted by the European Commission in its final report [11].

But this ADI is incorrect. The German authorities arrived at this ADI by excluding certain studies from the ADI process. First, they excluded the mid-term teratogenicity studies on rabbits discussed above, on the grounds that only long-term studies should be used to set safe chronic exposure levels. Second, they claimed that the most sensitive species for chronic exposure was the rat [50], providing another reason to exclude the rabbit teratogenicity studies.

However, while mid-term studies are generally discounted in ADI calculations because they are considered less sensitive than long-term studies, in this case, the mid-term rabbit studies found toxic effects at lower doses than the long-term studies in rats. Therefore, the mid-term rabbit studies were found to be more sensitive, and the rabbit was a more appropriate species. These data clearly indicate that the rabbit studies should have been used to set the ADI.

The exclusion of the toxicity studies in rabbits has introduced significant bias into the data used by the German regulators to calculate the ADI. The German authorities cited as their starting point for establishing the ADI a LOAEL of 60 mg/kg bw/d from a two-year study in rats, which found significant toxicity at that level (Suresh et al., 1996). This was stated to be the lowest dose at which toxicity was observed. They then identified the highest NOAEL below that level – 31 mg/kg bw/d – as the one from which the ADI should be calculated. Applying

the usual 100-fold safety factor, the German regulators proposed an ADI for glyphosate of 0.3 mg/kg bw/d [49].

The German authorities ignored the LOAEL of 20 mg/kg identified by Suresh et al. in rabbits, a value three times lower than their chosen LOAEL of 60 mg/kg bw/d [51].

The reason given by the German regulators for not adopting the 20 mg/kg LOAEL (Suresh et al., 1993) for setting the acceptable operator (applicators') exposure level (AOEL), is that it is a mid-term rather than long-term experiment and therefore more suitable for setting this type of level [52].

However, we propose that given the greater sensitivity of the rabbit model system to glyphosate exposure, the LOAEL of 20 mg/kg bw/d (Suresh et al., 1993) should have been the starting point for the ADI and for the applicators' AOEL. Indeed, this study found no NOAEL, as even the lowest dose produced toxic effects [51]. If the LOAEL of 20 mg/kg were used, employing the same procedure as the German regulators, the highest NOAEL below this dose from their approved list of studies is 10 mg/kg [49]. Applying the customary 100-fold safety factor to this value results in a more objectively accurate ADI of 0.1 mg/kg bw/d, one-third of the ADI suggested by the German authorities and subsequently adopted by the EU Commission.

The ADI According To Peer-Reviewed Studies

Two mammalian toxicological studies suggest that the LOAEL for glyphosate should be even lower than the ADI of 0.1 mg/kg bw/d that we derive from the industry-sponsored studies.

Romano et al. (2010) found that Roundup is a potent endocrine disruptor and disturbed the reproductive development of rats with exposure during puberty. Adverse effects, including delayed puberty and reduced testosterone production, were found at all dose levels, including the LOAEL of 5 mg/kg. There was a clear dose-response relationship [53].

Benedetti et al. (2004) found that Glyphosate-Biocarb caused "irreversible" damage to rat liver cells, including at the LOAEL of 4.87 mg/kg, with a clear dose-response relationship [54].

No dose below these LOAELs was tested in these two studies [53,54], so the NOAEL will be lower. Hypothetically, if the NOAEL were conservatively assumed to be 2.5 mg/kg bw/d, applying the 100-fold safety margin would result in an ADI of 0.025 mg/kg bw/d. This is twelve times lower than the ADI proposed by the German regulators, which is currently in force in the EU and used as a basis for the maximum residue limit for food and feed.

These studies used a species (rats) and exposure route (oral) that are accepted by industry, EU regulators and the Organisation for Economic Cooperation and Development (OECD). They tested specific glyphosate formulations, so it is not known whether their findings can be extrapolated to other formulations. However, this raises the crucial question of why formulations are approved on the basis of industry tests on, and a regulatory assessment of, only the isolated ingredient, glyphosate.

Papers Defending Glyphosate's Safety

In their rebuttal of Paganelli et al.'s study, the authors from Monsanto/Dow/Syngenta state:

The GLP [Good Laboratory Practice] studies that Paganelli et al. infer as untrustworthy "industry-funded studies" have been

exhaustively reviewed by multiple government scientific regulators, often comprised of academic expert scientists and all of which have strongly supported the conclusions put forth in those studies. Glyphosate does not cause adverse reproductive effects in adult animals or birth defects in offspring of these adults exposed to glyphosate, even at very high doses [16].

Given the evidence we present here from both academic and industry-sponsored studies, this argument is unconvincing. The data clearly show that glyphosate does cause adverse reproductive effects and malformations in laboratory animals.

Even if one accepts the position proposed by Monsanto/Dow/Syngenta, that only studies conducted according to GLP should be considered, this argument does not stand up to scrutiny, as some of the studies in the industry dossier on glyphosate are too old to utilize GLP [55].

Williams et al. (2000), in a paper that is frequently cited as evidence of the safety of Roundup and glyphosate, also cite the GLP status of industry studies to back their claim that glyphosate is not a reproductive toxin. However, some of the studies that they cite are, in fact, non-GLP: for example, Schroeder (1981) and Tasker (1980) [55]. Moreover, they fail to cite other studies from the same industry dossier – Suresh (1993), Brooker (1991), and Bhide and Patil (1989) [56], which found teratogenic effects from glyphosate, as detailed above.

It is important to note that GLP is not a measure of scientific reliability or validity, but a set of laboratory management rules instituted by regulators in the 1970s and 1980s to combat fraud in industry testing. Interestingly, the move to GLP standards was prompted by a high-profile case of fraud involving toxicological tests on glyphosate for regulatory purposes conducted by a laboratory under contract to Monsanto in late 1970s. However, the implementation of GLP failed to prevent a second major case of fraud, which came to light in the 1990s. This case also involved glyphosate at a different laboratory under contract to Monsanto, but this time involved residue tests [57,58]. While Monsanto said it later replaced the fraudulent tests [59], this history shows that industry-sponsored testing can be prone to fraud and that GLP cannot be assumed to prevent it.

Both Williams et al. (2000) and the authors from Monsanto/Dow/Syngenta cite World Health Organisation (WHO) reports in support of glyphosate's safety [60,56,16]. However, the WHO relies on data from industry studies [60], which, as shown above, in fact provide evidence of teratogenicity.

In addition, the study by Williams et al. (2000) was co-authored by Ian C. Munro, whose affiliation was listed as the chemical industry consulting firm Cantox [56]. Cantox states that its mission is to “protect client interests while helping our clients ... bring products to market” [61]. Williams et al. published their paper in the journal *Regulatory Toxicology and Pharmacology*, which was investigated by a US Congressional Committee in 2008 over its industry sponsorship in relation to its role in the FDA's decision allowing the endocrine-disrupting chemical bisphenol A in infant formula and other foods [62,63].

A Monsanto-funded review by Williams et al. (2012), co-authored by two representatives of the chemical industry consulting firm Exponent, argued for the unreliability of Dallegrave's study (2003) partly on the claimed basis that the malformations found were artefacts of histopathological fixation and processing [64]. But these hypothetical arguments could be countered by the clear dose-response relationship

(as required by regulators) found by Dallegrave [5]. Williams et al. also argued that the malformations were only “signs of a developmental delay that correct themselves within a brief period” [64]. The authors failed to provide citations of any experimental evidence upon which this claim was based and whether the malformations would indeed “correct themselves” without resulting in lasting damage to the developing central nervous system and other organs and systems. Thus this assertion remains unsubstantiated.

The argument used by Williams et al. (2012), is similar to the German authorities' redefinition of a malformation as a “developmental variation” [44]. Indeed, in a discussion of an unpublished mammalian toxicology study on glyphosate (IRDC, 1980a), Williams et al. followed the German authorities in defining the observed unossified sternebrae in treated rats as not a malformation but “a variation, possibly related to the reduced foetal weights and a developmental delay” [64].

With regard to Paganelli et al.'s study [1], Williams et al. (2012) stated that the glyphosate solution tested was not pH-adjusted and thus the malformations “may have been due to the acidic nature of the test compound” [64]. However, this hypothetical argument is spurious since at the dilutions used, the pH of the buffered test solution was not changed by the addition of herbicide.

BVL's Response to Dallegrave et al.

In its response to Paganelli et al. [1], BVL dismissed Dallegrave et al.'s study, which found malformations in the offspring of rats treated with Roundup, on the basis that “there were no craniofacial malformations” [13]. But this is a misrepresentation of Dallegrave's study, which stated, “The most frequent skeletal alterations observed were incomplete skull ossification and enlarged fontanel[le]” [5]. Both are craniofacial malformations. Therefore, contrary to BVL's assertion, Dallegrave et al.'s study provides clear evidence that a glyphosate herbicide can cause craniofacial malformations.

Moreover, by focusing on craniofacial malformations, BVL ignored the broad range of malformations associated with disturbances in the retinoic acid signalling pathway during development, which were found from glyphosate exposure by Dallegrave et al. [5], Paganelli et al. [1] and in the industry studies (see above). For example, a malformation found by Dallegrave et al. in a dose-dependent relationship was “caudal vertebrae: absent” [5]. This malformation is associated with the retinoic acid signalling pathway. Exposure of mouse embryos to retinoic acid at a similar period of development has been found to produce agenesis of caudal vertebrae, caused by the down-regulation of posterior Hox genes [20].

Reports and Studies from South America

Paganelli et al. stated that they were prompted to conduct their study by reports and studies indicating high rates of human birth defects in regions of South America dedicated to growing GM Roundup Ready soy [1].

For example, an epidemiological study carried out in Itapua, Paraguay, found a higher rate of malformations in the offspring of women exposed in pregnancy to pesticides, compared with controls. The malformations observed included craniofacial defects, anencephaly, microcephaly, hydrocephalus, myelomeningocele, cleft palate, anotia, polydactyly, syndactyly, and congenital heart defects [65].

Many of these malformations are of the same type as those observed by Paganelli et al. in frogs and chickens, and are associated

with the retinoic acid pathway. The authors do not mention glyphosate, and most agrochemical applications use mixtures of pesticides, so a sole causative agent cannot be identified. However, Itapua is an area of intensive Roundup Ready soy cultivation [66].

A study commissioned by the provincial government of Chaco, Argentina, a region of intensive GM soy production, showed a threefold increase in birth defects in the province and a fourfold increase in cancer in the locality of the agricultural town of La Leonesa in the last decade, coinciding with the expansion of GM soy and the associated application of pesticides. The authors named glyphosate as a pesticide of concern and noted that complaints from residents were highest in regions where GM crops are planted [67].

A study of birth defects in seven regions of Argentina found that Cordoba, an area of intensive planting of GM soy where pesticides are heavily used, had a higher incidence of spina bifida, microtia, cleft lip with cleft palate, polycystic kidney, postaxial polydactyly and Down's syndrome than other regions [68]. Many of these defects are of the type associated with disturbances in the retinoic acid signalling pathway, though it is not possible to identify a sole causative agent.

Epidemiological Studies in North America

Epidemiological studies carried out in North America show an association between exposure to glyphosate herbicides and adverse reproductive and developmental outcomes. In Canada, the Ontario Farm Family Health Study found a higher than normal rate of miscarriages and pre-term deliveries associated with glyphosate exposure [40,69]. An epidemiological study carried out in the USA found that the children of pesticide applicators exposed to glyphosate herbicides had an increased incidence of ADHD (attention deficit hyperactivity disorder) [70]. The finding suggested that glyphosate herbicide impacts neurological development.

Rull et al. provided evidence of an association between maternal exposure to glyphosate herbicides and anencephaly, a type of neural tube defect, as well as with neural tube defects (NTDs) in general [71,72]—consistent with retinoic acid-linked teratogenicity. The study found that maternal glyphosate herbicide exposure was associated with anencephaly using one type of analytical model (polytomous conventional multiple pesticide model), but not with another (hierarchical polytomous or single pesticide model).

The data showed modest associations between glyphosate and NTDs for both single and multiple pesticide models, with an odds ratio (OR) of 1.5 for both. For the hierarchical model the OR was 1.4. The authors' criteria for significant effects were that the OR should be greater than or equal to 1.4 and the lower limit of the confidence interval (CI) should be greater than or equal to 0.9. The OR requirement was met for glyphosate and NTDs using both models, but both models delivered CIs of 0.8, just below the cut-off value [71,72].

These results indicate a modest association between glyphosate herbicide exposure and NTDs and are in disagreement with the interpretation put forward by Williams et al. that the data shows “no effect” on NTDs. Williams et al. disagree with Rull et al.'s classification of glyphosate as an organophosphate [64], although chemically, it falls into that category of compounds.

Some studies that Williams et al. (2012) cite in their review in defence of the safety of glyphosate herbicides are unpublished industry-sponsored studies [64]. It should be noted, however, that the industry teratogenicity studies examined glyphosate and not the commercial

herbicide formulations, which are the substances under examination in epidemiological and most laboratory studies from the peer-reviewed literature. Crucially, these are also the substances to which humans are exposed. Studies have found that, although glyphosate itself is toxic, the formulations are more toxic than glyphosate alone [32,33,73,74]. Even the industry-sponsored studies on glyphosate alone show cause for concern, as shown above.

Genotoxicity of Glyphosate

While the EU Commission's 2002 review report on glyphosate concludes that it is “not genotoxic” [11], it is difficult to understand how this position can be maintained. Studies indicate that glyphosate herbicides are genotoxic and thus have the potential to increase the risk of birth defects and cancer. Cytogenetic monitoring of crop sprayers in Cordoba, Argentina revealed that the number of chromosomal aberrations in peripheral blood cells was significantly higher in the exposed group in comparison to the unexposed group. The pesticides most commonly used by the exposed group were glyphosate, cipermetrine, and atrazine [75].

An epidemiological study on Ecuadorian populations showed that people exposed to aerial glyphosate spraying showed a higher degree of DNA damage than a control population living 80 km away [76]. Mañás et al. found that glyphosate was genotoxic in the comet assay in Hep-2 cells and in the micronucleus test at 400 mg/kg in mice [77].

Glyphosate herbicides and glyphosate's main metabolite, AMPA, altered cell cycle checkpoints in sea urchin embryos by interfering with the DNA repair machinery [78-80]. The failure of cell cycle checkpoints is known to lead to genomic instability and cancer in humans. Glyphosate and AMPA have also been found to cause irreversible damage to DNA that may increase the risk of cancer [77,81]. AMPA damaged DNA in human cells at doses of 2.5-7.5mM and caused chromosomal breaks at 1.8mM [81].

The surfactants and other adjuvants in glyphosate formulations enhance the toxic effects of glyphosate, as they enable it to penetrate more easily through the cell membrane [79,82]. The adjuvants alone are also toxic [73].

Farm Family Exposure Study

The Monsanto/Dow/Syngenta authors cited the Farm Family Exposure Study (FFES) [83], as evidence that the doses used by Paganelli et al. and the suggestion of a link between glyphosate herbicide exposure and birth defects in Argentina are unrealistic [16]. The FFES measured urinary glyphosate concentrations for farmers, their spouses, and their children. The study concluded that the maximum systemic dose to spouses in the FFES was only 0.04 µg/kg body weight, with more than 95% of the spouse exposures below the limit of detection [83]. The Monsanto/Dow/Syngenta authors stated that this exposure scenario was “similar” to that of the populations in Argentina and other soy-producing regions of South America that were the focus of concern in Paganelli et al.'s study [16].

However, it is difficult to envisage how these two scenarios are similar. The US-based FFES measured urinary glyphosate concentrations the day before, the day of, and for three days following a single glyphosate application, which was carried out by tractor and boom sprayer. In the US, it is usual for farmers to conduct spraying from the relatively protected environment of an enclosed air-conditioned cab. People living in South American GM soy-producing regions are exposed not once but frequently during the growing season and application is often carried out from the air, leading to problems of drift.

Moreover, any evaluation of the effects of pesticide exposures must take into consideration the effects of repeated and continuous exposures. Bolognesi (2003) found that chromosomal damage caused by pesticides was temporary in short or time-limited exposures but cumulative in continuous exposures to agrochemicals [84].

The FFES authors acknowledged that the nature of their study may have led participating farmers to take extra care in their work. Therefore it may not have reflected real conditions, even in the US (a representative of the study was present with the farmer at the time of application). Also, the FFES was sponsored by members of the pesticide industry: Bayer, Dow, DuPont, FMC, Monsanto, Syngenta, and the American Chemistry Council. One author, Acquavella, was an employee of Monsanto; another was an employee of the industry consulting firm, Exponent. These links with the pesticide industry create a risk of bias.

For these reasons, the FFES may not reflect realistic conditions. Mage (2006) stated in a critique of the FFES that a study that randomly and frequently assesses glyphosate burdens in farm families over a long period of time would provide a more realistic assessment of exposure [85].

Our concerns are supported by another study, which is not mentioned by the authors of the Monsanto/Dow/Syngenta rebuttal. In a study investigating pesticide exposure in farm and non-farm families in Iowa, USA, Curwin et al. (2007) found that 75% of farmers, 67% of wives, and 81% of farmers' children were carrying urinary burdens of more than 900 ppb of glyphosate (0.9 mg/kg bw) [86]. In contrast, the FFES reported average urinary burdens of glyphosate ranging from 1 to 6.4 ppb on different days of the study for farmers, and with averages close to 0 ppb for wives and children (less than 25% of subjects were reported to have any detectable urinary glyphosate burden) [83].

Court Cases on Glyphosate Herbicide Exposure

The safety of glyphosate herbicides has been successfully challenged in several court cases. In New York in 1996, a court ruled that Monsanto was no longer allowed to market Roundup as safe, non-toxic, biodegradable or environmentally friendly [87]. In France in 2007, Monsanto was forced to withdraw advertising claims that Roundup was biodegradable and leaves the soil clean after use [88]. In March 2010, in a case brought by residents, a court in Santa Fe province, Argentina instituted a regional ban on the spraying of glyphosate and other agrochemicals in populated areas on grounds of "severe damage to the environment and to the health and quality of life of the residents" [89].

In June 2012 criminal charges were brought by affected residents against two soy producers and a crop-spraying airplane pilot, in a case heard by a court in Cordoba, Argentina. Plaintiffs charged the defendants with malicious contamination over the spraying of glyphosate and other agrochemicals in Ituzaingó, an area on the outskirts of Cordoba reportedly characterized by a high incidence of cancer and birth defects [90].

Relevance of Different Exposure Routes

BVL and Monsanto/Dow/Syngenta dismissed Paganelli et al.'s study on the grounds that it used inappropriate exposure routes. They object to injection and culture on the grounds that they are "highly artificial", "do not reflect human exposure" [13], and are "irrelevant" [16], to human risk assessment. This argument is also used by Williams et al. (2012) in defence of the safety of glyphosate [64].

The standard that is being invoked is not named but is likely to be the OECD standardised protocols for industry studies performed for regulatory purposes, which prefer oral, dermal or inhalation exposure routes [91].

OECD guidelines exist to guide industry on how to conduct standardised tests performed for regulatory purposes, but it is not credible to suggest that they represent the only valid or the most scientifically rigorous route to acquiring information about a chemical's toxicity.

In the case of Paganelli et al.'s study [1], injection of the treated group with glyphosate and of the control group with water clarified that only one substance-glyphosate-could have caused the malformations. The absence of malformations in the water-injected controls showed that the trauma of injection did not cause the malformations.

Two studies comparing oral dosing with injection presented findings that challenge assumptions about different exposure routes:

- A study comparing the effects of bisphenol A (BPA) administered to rats by oral dosing and injection found that after two hours, the level of active BPA in the blood was the same between orally dosed and injected groups. Both exposure routes resulted in the same pre-cancerous toxic effects on the prostate seven months after exposure. The study concluded that the internal received dose, not route of exposure, is the critical factor, and that therefore, the injection exposure route should be acceptable for human risk assessment [92,93].
- A study comparing the toxicokinetics of glyphosate administered to rats by oral dosing and injection found that when given orally, glyphosate was more slowly absorbed but took longer to clear from blood, leading to the possibility that it could be distributed to the tissues, causing systemic toxic effects [94]. The oral route is favoured by industry and regulators on the claimed grounds that it better reflects real human exposures. Thus, based on this study, experiments using injection could be assumed to result in less toxic effects than those using oral methods.

While doses received by different tissues may vary according to exposure route, this should be tested and not assumed. It seems critically important to conduct biomonitoring studies on exposed populations to discover how much glyphosate and its main metabolite, AMPA, is present in tissues and to investigate the potential for bioaccumulation.

An *in vitro* study on human buccal cells attempted to mirror human exposures to glyphosate herbicide through inhalation. The study found that glyphosate and Roundup caused DNA damage in the cells after a single 20-minute exposure at a dose corresponding to a 450-fold dilution of the concentration used in agriculture. Roundup was more toxic than glyphosate alone. The authors concluded that inhalation may cause DNA damage in exposed individuals and that the DNA damage was caused directly by the substances instead of being an indirect result of cell toxicity [95].

While the Monsanto/Dow/Syngenta authors condemn *in vitro* methods as "unvalidated", this value judgement only raises the question: validated by whom? We interpret this statement to mean that these *in vitro* tests do not conform to OECD standardised protocols for industry toxicological studies. However, outside the narrow context of industry testing for regulatory purposes, such *in vitro* tests are an important tool. For example, in the pre-clinical phase of pharmaceutical drug development, if a potential drug gives a positive micronucleus test

in vitro, then development is discontinued. Also, such *in vitro* tests add valuable evidence to findings from laboratory in vivo and human epidemiological studies. Regarding glyphosate and Roundup, studies of all these types suggest that both substances are genotoxic and have toxic effects on development and reproduction.

Unrealistically High Doses?

In their response to Paganelli et al., the Monsanto/Dow/Syngenta authors argue that the researchers used “inappropriately high” and “unrealistic” doses, far higher than the already high doses that have been shown in other studies not to cause malformations [16].

Considering first Paganelli et al.’s frog embryo injection experiments, calculations based on Monsanto/Dow/Syngenta’s own paper show that the doses were not inappropriately high. The Monsanto/Dow/Syngenta authors stated that a 400 mg/kg dose of glyphosate, delivered through feeding, results in a blood concentration of 4.6 µg/ml. Animal studies typically use between 50 and 500 mg/kg bw/d doses. Making a linear extrapolation (as the Monsanto/Dow authors do for other purposes), a 50 mg/kg dose should result in a blood concentration of 0.575 µg/ml, or 575 µg/L. Therefore, the range of blood concentrations achieved in animal studies would be in the range of 575–5750 µg/L. Clearly, the concentrations achieved in the frog embryos (690–950 µg/L) are comparable to the blood concentrations typically achieved in animal feeding studies.

Regarding Paganelli et al.’s frog embryo culture experiments, the Monsanto/Dow/Syngenta authors stated that the concentrations used were 9–15 times greater than the acute LC50 value for frog embryos of the same species. Monsanto/Dow cites as its authority for this argument a study by Edginton et al. [96]. However, Edginton used a different glyphosate formulation, with a potentially different LC50 value. Moreover, the low mortality rate found by Paganelli et al. counters the Monsanto/Dow/Syngenta authors’ claim that the doses used were even close to the LC50 value.

Regarding Paganelli et al.’s experiments with chicken eggs, using the Monsanto/Dow/Syngenta authors’ own estimate that 20 µL of a 1/4500 dilution of glyphosate-formulated product translates to 2 µg glyphosate injected into the egg, and assuming that the volume of a chicken egg is approximately 35 ml, the actual concentration of glyphosate within the egg would be 57 µg/L. This is much lower than the blood concentrations of glyphosate that would be expected in animal toxicity studies (575–5750 µg/L, see above), according to Monsanto/Dow’s own calculation methods.

Further countering the claim that Paganelli et al. used unrealistically high doses or doses higher than the LC50 value is new, as yet unpublished data obtained by the same researchers. In these culture experiments with embryos of *Xenopus laevis*, the same methodology was followed as in the original culture experiments detailed in Paganelli et al. [1] A different commercial formulation of Roundup was used (Gleba from Gleba S.A., instead of the Roundup Classic used in the original experiments) and batches of embryos were cultured in progressively lower dilutions. The same malformations as were observed in the original experiments were reproduced in a dose-dependent manner, even at dilutions of 1/500,000 (4.30 µM). This dilution produced developmental abnormalities in 17% of the embryos, with no lethality [97].

Conclusion

Studies published in the peer-reviewed scientific literature have raised major concerns regarding the potential for glyphosate and its

commercial formulations to cause birth defects and other reproductive problems. In addition, a debate has emerged over the reported effects on human health of herbicide application in regions that produce GM glyphosate-tolerant crops and about the safety of food and feed produced from these crops.

Regulatory authorities and industry affiliates have defended the use of glyphosate largely by citing the industry-sponsored toxicological tests conducted for regulatory purposes, which they claimed showed no evidence of teratogenicity. However, the German authorities’ draft assessment report revealed that even these industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity. Many of the malformations observed in these studies are of the type associated with the retinoic acid signalling pathway. Paganelli et al. [1] showed that this was the mechanism through which glyphosate and Roundup exercise their teratogenic effects.

It is noteworthy that these industry tests were commissioned by the same companies that stand to profit from regulatory authorization. Regrettably, this system possesses an inherent risk of bias and makes it especially important that the regulatory assessment is rigorous. Yet in the EU, the evidence suggests that this was not the case. The significance of clear teratogenic effects of glyphosate in rabbits and rats found in tests commissioned by industry were minimized by German regulators. A scientifically rigorous assessment was further impeded by the outdated design of the standard tests, which are not sufficiently sensitive to detect effects from realistic exposures. As a result, the German authorities suggested, and the EU adopted, an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans.

Another relevant factor is that the industry teratogenicity tests were on glyphosate, the presumed active ingredient of the herbicide, and not on the herbicide formulations as sold and used, even though studies indicate that the formulations are more toxic for certain endpoints than glyphosate alone.

A substantial body of evidence demonstrates that glyphosate and Roundup cause teratogenic effects and other toxic effects on reproduction, as well as genotoxic effects. From an objective scientific standpoint, attempts by industry and government regulatory bodies to dismiss this research are unconvincing and work against the principle that it is the responsibility of industry to prove that its products are safe and not the responsibility of the public to prove that they are unsafe. The precautionary principle would suggest that glyphosate and its commercial formulations should undergo a new risk assessment, taking full account of the entirety of the peer-reviewed scientific literature as well as the industry-sponsored studies. Experience to date suggests that the new risk assessment should be conducted with full public transparency by scientists who are independent of industry.

Disclaimer

The opinions expressed are those of the individual authors and do not reflect the policies of organizations with which they are associated.

References

1. Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem Res Toxicol 23: 1586-1595.
2. Jayawardena UA, Rajakaruna RS, Navaratne AN, Amerasinghe PH (2010) Toxicity of agrochemicals to common hourglass tree frog (*Polypedates cruciger*) in acute and chronic exposure. Int J Agric Biol 12: 641-648.
3. Relyea RA (2012) New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. Ecological Applications 22: 634-647.

4. Lajmanovich RC, Sandoval MT, Peltzer PM (2003) Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. *Bull Environ Contam Toxicol* 70: 612-618.
5. Dallegre E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, et al. (2003) The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. *Toxicol Lett* 142: 45-52.
6. GMO Compass (2012) Soybeans. Genius GmbH.
7. Bindraban PS, Franke AC, Ferrar DO, Ghersa CM, Lotz LAP, et al. (2009) GM-related sustainability: Agro-ecological impacts, risks and opportunities of soy production in Argentina and Brazil. *Plant Research International*, Wageningen, The Netherlands.
8. Branford S (2004) Argentina's bitter harvest. *New Scientist*, 17 April.
9. Mesnage R, Clair E, Gress S, Then C, Székács A, et al. (2012) Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *J Appl Toxicol* 15.
10. Food and Agriculture Organization (FAO) (2005) Pesticide residues in food-2005: Evaluations Part I: Residues (S. 477).
11. European Commission Health & Consumer Protection Directorate-General (2002) Review report for the active substance glyphosate.
12. Antoniou M, Habib M, Howard CV, Jennings RC, Leifert C, et al. (2011) Roundup and birth defects: Is the public being kept in the dark? *Earth Open Source*. 1-52.
13. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) (2010) Glyphosate – Comments from Germany on the paper by Paganelli, A. et al.: "Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling". Braunschweig, Germany.
14. Dalli J (2011) Answer given by Mr Dalli on behalf of the Commission, Parliamentary questions, European Parliament, Brussels. 10.
15. European Commission (2010) Directive 2010/77/EU of 10 November 2010 amending Council Directive 91/414/EEC as regards the expiry dates for inclusion in Annex I of certain active substances. *Official Journal of the European Union*.
16. Saltmiras D, Bus JS, Spanogle T, Hauswirth J, Tobia A, et al. (2011) Letter to the editor regarding the article by Paganelli et al. *Chem Res Toxicol* 24: 607-608.
17. Carrasco AE (2011) Reply to the letter to the editor regarding our article (Paganelli et al., 2010). *Chem Res Toxicol* 24: 610-613.
18. European Commission (1998) Glyphosate. Reasoned statement of the overall conclusions drawn by the rapporteur member state, in: Rapporteur member state, Germany (1998) Monograph on Glyphosate, 1: 9.
19. Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, et al. (1985) Retinoic acid embryopathy. *N Engl J Med* 313: 837-841.
20. Kessel M (1992) Respecification of vertebral identities by retinoic acid. *Development* 115: 487-501.
21. Huang FJ, Wu TC, Tsai MY (2001) Effect of retinoic acid on implantation and post-implantation development of mouse embryos in vitro. *Hum Reprod* 16: 2171-2176.
22. Malpel S, Mendelsohn C, Cardoso WV (2000) Regulation of retinoic acid signaling during lung morphogenesis. *Development* 127: 3057-3067.
23. Wilson JG, Roth CB, Warkany J (1953). An analysis of the syndrome of malformation induced by maternal vitamin A deficiency: Effects of restoration of vitamin A at various times during gestation. *Am. J. Anat.* 92: 189-217.
24. Shenefelt RE (1972) Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage at treatment. *Teratology* 5: 103-118.
25. Dickman ED, Thaller C, Smith SM (1997). Temporally-regulated retinoic acid depletion produces specific neural crest, ocular and nervous system defects. *Development* 124: 3111-3121.
26. Paumgarten FJ (2010) Influence of maternal toxicity on the outcome of developmental toxicity studies. *J Toxicol Environ Health A* 73: 944-951.
27. Dallegre E, Mantese FD, Oliveira RT, Andrade AJ, Dalsenter PR, et al. (2007) Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Arch Toxicol* 81: 665-673.
28. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, et al. (2003) Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 111: 994-1006.
29. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr et al. (2012) Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocr Rev* 33: 378-455.
30. Goldsmith JR, Kordysh E (1993) Why dose-response relationships are often non-linear and some consequences. *J Expo Anal Environ Epidemiol* 3: 259-276.
31. Vom Saal FS, Hughes C (2005) An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* 113: 926-933.
32. Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005) Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ Health Perspect* 113: 716-720.
33. Axelrad JC, Howard CV, McLean WG (2003) The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. *Toxicology* 185: 67-78.
34. Haseman JK (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ Health Perspect* 58: 385-392.
35. Hardisty JF (1985) Factors influencing laboratory animal spontaneous tumor profiles. *Toxicol Pathol* 13: 95-104.
36. Cuffe RL. The inclusion of historical control data may reduce the power of a confirmatory study. *Stat Med* 30: 1329-1338.
37. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 15-17.
38. EU Commission (1999) Glyphosate: Comments from Pesticides Safety Directorate, UK, on EC Monographs for Glyphosate and Glyphosate Trimesium: Glyphosat 5: 25.
39. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 18.
40. Savitz DA, Arbuckle T, Kaczor D, Curtis KM (1997) Male pesticide exposure and pregnancy outcome. *Am J Epidemiol* 146: 1025-1036.
41. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 19.
42. EU Commission (1999) Glyphosate: Comments from Pesticides Safety Directorate, UK, on EC Monographs for Glyphosate and Glyphosate Trimesium: Glyphosat 5: 26.
43. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 19, 10.
44. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 13.
45. Krieger RI (2001) Handbook of Pesticide Toxicology, Vol. I: Principles. Academic Press, San Diego, CA, p. 1185.
46. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 12.
47. EU Commission ECCO Panel (1999) Discussion ECCO Peer Review Meeting: Glyphosate (Hb), Appendix 1, Glyphosat 3: 30.
48. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 41.
49. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 43.
50. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 42.
51. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 15-17, 42-44.
52. Rapporteur member state, Germany (1998) Monograph on Glyphosate. Annex B-5: Toxicology and Metabolism: Volume 3-1, Glyphosat 5: 44.
53. Romano RM, Romano MA, Bernardi MM, Furtado PV, Oliveira CA (2010) Prepubertal exposure to commercial formulation of the herbicide Glyphosate alters testosterone levels and testicular morphology. *Arch Toxicol* 84: 309-317.

54. Benedetti AL, Vituri C de L, Trentin AG, Domingues MA, Alvarez-Silva M (2004) The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb. *Toxicol Lett* 153: 227-232.
55. The non-GLP status of these studies is shown in: Rapporteur member state, Germany (1998) Monograph on Glyphosate. List of Tests and Studies.
56. Williams GM, Kroes R, Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul Toxicol Pharmacol* 31: 117-165.
57. Cox C (1998) Glyphosate (Roundup). *J Pesticide Ref* 18: 3-17.
58. Novak RA (2001) The long arm of the lab laws. *Today's Chemist at Work* 10: 45-46.
59. Monsanto (2005). Backgrounder: Testing fraud: IBT and Craven Labs.
60. World Health Organization and Food and Agriculture Organization (2004) Pesticide residues in food – 2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. FAO Plant Production and Protection Paper 178: 98-103.
61. Cantox (2012) Cantox Health Sciences International.
62. Layton L (2008) Studies on chemical in plastics questioned. *Washington Post*.
63. Dingell JD, Rep. (D-Mich.) (2008) Letter to Jack N. Gerard, president and CEO, American Chemistry Council.
64. Williams AL, Watson RE, DeSesso JM (2012) Developmental and reproductive outcomes in humans and animals after glyphosate exposure: A critical analysis. *J Toxicol Environ Health B Crit Rev* 15: 39-96.
65. Benítez-Leite S, Macchi ML, Acosta M (2009) Malformaciones congénitas asociadas a agrotóxicos [Congenital malformations associated with toxic agricultural chemicals]. *Arch Pediatr Urug* 80: 237-247.
66. Gray L (2011) GM soy: the high cost of the quest for "green gold". *The Telegraph* (UK).
67. Provincial Research Commission Water Pollutants, First Report [First report], Resistencia, Chaco, Argentina (2010).
68. Campana H, Pawluk MS, Lopez Camelo JS (2010) [Births prevalence of 27 selected congenital anomalies in 7 geographic regions of Argentina]. *Archivos Argentinos de Pediatría* 108: 409-417.
69. Arbuckle TE, Lin Z, Mery LS (2001) An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. *Environmental Health Perspectives* 109: 851-857.
70. Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, et al. (2002) Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. *Environ Health Perspect* 110: 441-449.
71. Rull RP, Ritz B, Shaw GM (2004) Neural tube defects and maternal residential proximity to agricultural pesticide applications. *Epidemiology* 15: S188.
72. Rull RP, Ritz B, Shaw GM (2006) Neural tube defects and maternal residential proximity to agricultural pesticide applications. *Am J Epidemiol* 163: 743-753.
73. Benachour N, Séralini GE (2009) Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol* 22: 97-105.
74. Dallegrove E, Mantese FD, Dalsenter PR, Langeloh A (2002) Acute oral toxicity of glyphosate in Wistar rats. *Online J Vet Res* 1: 29-36.
75. Mañas F, Peralta L, Gorla N, Bosh B, Aiassa D (2009) Chromosomal aberrations in workers occupationally exposed to pesticides in Córdoba. *J Basic Appl Genet* 20: 9-13.
76. Paz-y-Miño C, Sánchez ME, Arévalo M, Muñoz, MJ, Witte T, et al. (2007) Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genetics and Molecular Biology* 30: 456-460.
77. Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, et al. (2009) Genotoxicity of glyphosate assessed by the Comet assay and cytogenetic tests. *Environ Toxicol Pharmacol* 28: 37-41.
78. Marc J, Mulner-Lorillon O, Belle R (2004) Glyphosate-based pesticides affect cell cycle regulation. *Biol Cell* 96: 245-249.
79. Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, et al. (2002) Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyclin B activation. *Chem Res Toxicol* 15: 326-331.
80. Marc J, Belle R, Morales J, Cormier P, Mulner-Lorillon O (2004) Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. *Toxicol Sci* 82: 436-442.
81. Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, et al. (2009) Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol Environ Saf Mar* 72: 834-837.
82. Haefs R, Schmitz-Eiberger M, Mainx HG, Mittelstaedt W, Noga G (2002) Studies on a new group of biodegradable surfactants for glyphosate. *Pest Manag Sci* 58: 825-833.
83. Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, et al. (2004) Glyphosate biomonitoring for farmers and their families: Results from the Farm Family Exposure Study. *Environ Health Perspect* 112: 321-326.
84. Bolognesi C (2003) Genotoxicity of pesticides: a review of human biomonitoring studies. *Mutat Res* 543: 251-272.
85. Mage DT (2006) Suggested corrections to the Farm Family Exposure Study. *Environ Health Perspect* 114: A633.
86. Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, et al. (2007) Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg* 51: 53-65.
87. Attorney General of the State of New York, Consumer Frauds and Protection Bureau, Environmental Protection Bureau (1996) In the matter of Monsanto Company, respondent. Assurance of discontinuance pursuant to executive law § 63(15). New York, Nov. False advertising by Monsanto regarding the safety of Roundup herbicide (glyphosate).
88. Agence France Presse (2007) Monsanto fined in France for "false" herbicide ads. *Organic Consumers Association*.
89. Romig S (2010) Argentina court blocks agrochemical spraying near rural town. *Dow Jones Newswires*. 17 March.
90. Magnani, R. (2012) Niños con malformaciones de nacimiento [Children with birth defects]. *Página 12*.
91. Organisation for Economic Cooperation and Development (OECD) (2009) OECD guideline for the testing of chemicals: Chronic toxicity studies. Adopted.
92. Prins GS, Ye SH, Birch L, Ho SM, Kannan K (2011) Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. *Reprod Toxicol* 31: 1-9.
93. Mackar R (2010) New BPA findings help fill research gaps. *Environmental Factor, NIEHS*, October.
94. Anadón A, Martínez-Larranaga MR, Martínez MA, Castellano VJ, Martínez M, et al. (2009) Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. *Toxicol Lett* 190: 91-95.
95. Koller VJ, Furrhacker M, Nersesyan A, Misik M, Eisenbauer M, et al (2012) Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol* 86: 805-813.
96. Edgington AN, Sheridan PM, Stephenson GR, Thompson DG, Boermans HJ (2004) Comparative effects of pH and Vision herbicide on two life stages of four anuran amphibian species. *Environ Toxicol Chem* 23: 815-822.
97. Carrasco AE (2012) Personal communication.