

Lab Study Establishes Glyphosate Link to Birth Defects

Dr. Mae-Wan Ho

ISIS, 4 October 2010

<http://www.i-sis.org.uk/glyphosateCausesBirthDefects.php>

*Developmental biologists traced glyphosate birth defects to key morphogen and signalling genes

*This report has been submitted to the US Environment Protection Agency and the European Food Safety Authority, please circulate widely to your political representatives and demand a worldwide ban on glyphosate herbicides

*Four-fold increase in human birth defects prompted lab study

It started in 2002, two years after the large scale introduction of Roundup Ready soybeans to Argentina [1]. People were reporting birth defects from exposure to glyphosate sprays during pregnancy; and the problem got worse.

In regions where glyphosate-based herbicides are used, specific neural defects and craniofacial malformations were reported. This prompted Prof. Andrés Carrasco, director of the Laboratory of Molecular Embryology at the University of Buenos Aires Medical School into action. He and his colleagues carried out a laboratory study on the effects of glyphosate on the development of frog embryos. They found the same kinds of abnormalities in frog embryos incubated with a 5 000 fold diluted solution of the Roundup herbicide [2]. The findings were so serious that Carrasco decided to release the results before publication [3] (Glyphosate Herbicide Could Cause Birth Defects Glyphosate Herbicide Could Cause Birth Defects, SiS 43), and in May 2009, the Environmental Lawyers Association of Argentina initiated a lawsuit to ban the herbicide.

In April 2010, the first official report commissioned by the State Government of Chaco documented a four-fold increase in both cancer and birth defects in the ten years of 2000 - 2009 [4].

These findings are the latest addition to a long chain of evidence linking glyphosate (and others herbicides) to serious health impacts at concentrations well below the level of recommended agricultural use. In the case of glyphosate herbicides, links to cancers, miscarriages and other reproductive toxicities, liver and cell toxicities, DNA damages, lethality to amphibians and endocrine disrupting action previously made, would more than justify a worldwide ban ([5] (Ban Glyphosate Herbicides Now, SiS 43); quite apart from simultaneous evidence of ecological/agronomic disasters caused by the herbicide ([6] Scientists Reveal Glyphosate Poisons Crops and Soil, SiS 47), while the spread of glyphosate resistant weeds and superweeds has rendered the GM crop and the herbicide practically useless ([7] GM Crops Facing Meltdown in the USA, SiS 46).

Lab findings in frog embryos parallel human abnormalities

In the first experiment, *Xenopus laevis* (the African 'clawed toad', which is really a frog) embryos

were incubated with high dilutions of a commercial glyphosate based herbicide (Roundup Classic, Monsanto). The embryos were exposed from the 2-cell stage with dilutions of the herbicide at 3 000-, 4 000-, and 5 000-fold (the most dilute equivalent to 430 mm of glyphosate). They found highly abnormal embryos in the regions of the head and central nervous system, and shortening of the anterior-posterior axis, even at the highest dilution. These led to deformities in the head cartilages at tadpole stage.

The second experiment indicated that the abnormalities were due to the herbicide glyphosate rather than the adjuvants in the Roundup formulation. The early frog embryos were injected with pure glyphosate, and they developed very similar defects. Injection of glyphosate into one cell at the two cell stage showed that the effects were restricted to the injected cell, with the non-injected cell acting as a control (see Fig 1).

Figure 1 Effect of glyphosate injection; left to right: control embryo not injected with glyphosate; embryo injected in one cells only; and embryo injected in both cells

The researchers then treated chick embryos with glyphosate-based herbicides and found similar defects. There was a gradual loss of head features including the future eyes, and the embryos ended up with microcephaly (small head). Embryonic defect traced to over active retinoic acid and expression of key developmental genes

To pin down the effect of glyphosate further, a reporter gene assay was carried out, which revealed that glyphosate treatment increased retinoic acid (RA) activity in the frog embryos. RA is an oxidized form of vitamin A well-known to have an important role in determining the anterior and posterior axis in embryonic development. That is why excess intake of vitamin A during pregnancy is advised against, as it can cause birth defects.

As consistent with glyphosate causing the malformations through increasing RA activity, the RA antagonist (Ro 41-5253) rescued (prevented) the teratogenic effect of glyphosate.

A previous study had indicated that women exposed during pregnancy to herbicides delivered offspring with congenital malformations, including microcephaly, anencephaly (no head), and cranial (head skeleton) malformations [8].

The increase in RA activity caused by glyphosate herbicides is also consistent with the decrease in expression of several genes in the abnormal embryos: Sonic hedgehog (Shh) signalling from the embryonic dorsal midline and *otx2*.

In humans, *shh* deficiency is associated with a holoprosencephaly syndrome (failure of forebrain development into two lobes), with a frequency of 1/250 of pregnancies and 1/10 000 of live births. Failure of Shh signalling is associated with head and face abnormalities in mice, zebrafish and the chick.

An excess of RA is also known to down regulate *otx2* expression in *Xenopus*, chicken and mouse embryos. *Otx2* in turn is necessary for the expression of *shh* in the ventral midbrain. All the evidence indicates that RA signalling, *otx2*, and *shh* are part of a genetic cascade critical for

the development of the brain and craniofacial skeleton. Glyphosate inhibits the anterior expression of *shh*, reduces the domain of *otx2*, prevents the subdivision of the eye field (see Fig. 1), and impairs craniofacial development.

Sublethal doses of the herbicide (430 mM of glyphosate in 1/ 5 000 dilution of the glyphosate based herbicide and injections leading to a final concentration of 8 to 12 mM of glyphosate in the injected side of the embryo were sufficient to induce serious disturbances in the expression of *otx2* and *shh* and other genes. These molecular phenotypes were correlated with a disruption of developmental mechanisms involving the neural crest, embryonic dorsal midline formation and head patterning.

Death and destruction by multiple poisoning

So how does glyphosate increase RA in the embryo to trigger the series of gene expression and developmental abnormalities?

RA activity is regulated by the degradation of RA by the CYP26 enzymes, these enzymes are members of the cytochrome P450 family (same as aromatase, which glyphosate inhibits). So it is possible that glyphosate increases RA by inhibiting the enzyme that degrades RA.

Actually, glyphosate is a generalized chelator (binder) of metal ions with systemic effects on many key enzymes that depend on multivalent ions (see [9] Glyphosate Tolerant Crops Bring Diseases and Death, SiS 47). As cytochromes are iron-containing heme-based enzymes, it would not be surprising if glyphosate inhibits RA degradation through binding the heme-iron. What is becoming clear is that glyphosate can poison crops, soils, wild life, livestock, human beings and the entire ecosystem in multiple systemic ways, and a global ban is long overdue.

References

1. Groundbreaking study shows Roundup link to birth defects, 16 September, 2010, http://www.gmwatch.org/index.php?option=com_content&view=article&id=12491
2. Paganelli A, Gnazzo V, Acosta H, Lopez SL and Carrasco AD. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, August 9. <http://pubs.acs.org/doi/abs/10.1021/tx1001749>
3. Ho MW. Glyphosate herbicide could cause birth defects. *Science in Society* 43, 36, 2009.
4. Chaco government report confirms link between glyphosate/agrochemicals and cancer/birth defects in Argentina
http://www.gmwatch.eu/index.php?option=com_content&view=article&id=12481:reports-official-report-confirms-correlation
5. Ho MW. Ban glyphosate herbicides now. Scientists confirm potent hormone disrupting effects. *Science in Society* 43, 34-35, 2009.
6. Ho MW. Scientists reveal glyphosate poisons crops & soil, GM meltdown continues. *Science*

in Society 47, 10-11, 2010.

7. Ho MW. GM crops facing meltdown in the USA. Science in Society 46, 24-27, 2010.

8. Benitez LS, Macchi MA and Acosta M. Malformaciones Congénitas asociadas a agrotóxicos. Arch Pediatr Drug 2009, 80, 237-47.

9. Ho MW and Cherry B. Glyphosate tolerant crops bring diseases and death. Science in Society 47, 12-15, 2010.