

## Detection of Glyphosate in Malformed Piglets

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### Summary

Glyphosate residues in different organs and tissues as lungs, liver, kidney, brain, gut wall and heart of malformed euthanized one-day-old Danish piglets (N= 38) were tested using ELISA. All organs or tissues had glyphosate in different concentrations. The highest concentrations were seen in the lungs (Range 0.4-80 µg/ml) and hearts (Range 0.15-80 µg/ml). The lowest concentrations were detected in muscles (4.4-6.4 µg/g). The detection of such glyphosate concentrations in these malformed piglets could be an allusion to the cause of these congenital anomalies. Further investigations are urgently needed to prove or exclude the role of glyphosate in malformations in piglets and other animals.

### Introduction

In spring of last year a Danish pig farmer brought 38 live borne but malformed one-day-old piglets into our laboratory because of extraordinary high percentages of malformations in piglets. It was reported an assumption about the possible causes of this incident. It was noticed that the rate of malformations increased to one out of 260 born piglets if sow feeds contain 0.87-1.13 ppm glyphosate (N-phosphonomethylglycine) in the first 40 days of pregnancy. In case of 0.25 ppm glyphosate in sow feeds one of 1432 piglets was malformed. These piglets showed different abnormalities as ear atrophy, spinal and cranial deformations, cranium hole in head and leg atrophy; in one piglet one eye was not developed, it had only a large one. Piglets without trunk, with elephant tongue, and female piglet with testes were also present. One malformed piglet showed a swollen belly and fore gut and hind gut were not connected (Figure 1). Different organs and tissues as lungs, liver, kidney, brain, gut wall and heart of malformed euthanized one-day-old Danish piglets were tested for glyphosate using ELISA [1]. Briefly, tissue samples were minced to small pieces (~0.25 cm). In relation to the ability to retain water, samples were diluted with distilled water (Braun, Germany). The specimens were heated at 100°C for 10 min, homogenized and frozen at -80°C for 8 h. Samples were carefully thawed at 40°C and centrifuged at 10.000 x g for 10 min. The supernatant was filtered with an ultra-centrifugal filter with a cut off of 3000 Da to remove proteins and peptides. Filtrates were centrifuged (10.000 x g) again at 20°C for 10 min and the supernatant was tested for glyphosate using ELISA kits (Abraxis, USA) according to the manufacturer's protocol.

Glyphosate residues were detected in the above mentioned tissues and organs of these piglets in different concentrations (Table 1). All organs or tissues had no significant differences in glyphosate concentration. The highest concentrations were seen in the lung (Range 0.4-80 µg/ml) and hearts (Range 0.15-80 µg/ml). The lowest concentrations were detected in muscles (4.4-6.4 µg/ml). It is postulated that glyphosate reaches the piglets through the placenta of their dams.

The predominant uses of glyphosate are for stubble management, pre-sowing weed control and pre-harvest application (desiccation) [2]. Glyphosate is also used for weed control in fields of genetically modified (GM) crops like soybean, rapeseed, corn, cotton, sugar beets, alfalfa, etc, where it is directly applied to the plants [3]. The rapidly

growing problem of glyphosate-resistant weeds is reflected in a steady increase in the rate of glyphosate used on crops. Stems, leaves and beans of glyphosate resistant soy are contaminated with glyphosate. Moreover, because of the extensive use of glyphosate, it is frequently detected in water, rain and air [4,5]. Recently, glyphosate residues were tested in urine and different organs of dairy cows as well as in urine of hares, rabbits and humans in different concentrations [6]. Glyphosate and its commercial herbicides severely affect embryonic and placental cells, producing mitochondrial damage, necrosis and programmed cell death with doses far below the used agricultural concentrations. Paganelli et al. [7] found congenital malformations in chicken embryos with glyphosate at a concentration of 8-12 µM glyphosate in the injected side. The molecular phenotypes were correlated with a disruption of developmental mechanisms involving the neural crest, embryonic midline formation and cephalic patterning induced by the active principal of glyphosate not by the adjuvants due to impairment of retinoid signaling. The authors gave an overview of reports of malformations in children of families living few meters from where this herbicide was sprayed. The risk of malformation in human embryos is very high when their mothers are contaminated at 2 to 8 weeks of pregnancy. The detected glyphosate concentrations in organs, gut walls and meat of these piglets suspect correlation to glyphosate. Daruich and co-workers [8] concluded that glyphosate causes various disorders both in the parent female and in the progeny. Paternal exposure to glyphosate is recognized to be a cause of birth defects by pesticide mediated alterations of germ cells [9,10].

In conclusion, glyphosate could reach the animals through food

	Minimum	Maximum	Mean ±SD
Lung (N=38)	0.15	80	7.7 ± 18
Liver (N=38)	0	29.25	2.1 ± 2.2
Kidney (N=38)	0.1	38	3.2 ± 1.8
Muscles (N=38)	4.4	6.4	4.9 ± 1.8
Brain (N=38)	0.4	19.7	3.1 ± 4.1
Intestin (N=12)	0.7	7.7	2.4 ± 19
Heart (N=8)	0.4	80	12.9 ± 29.8

<sup>1</sup>Glyphosate was detected using ELISA [1] and data are expressed as µg/g.

**Table 1:** Glyphosate residues in different organs of malformed piglets<sup>1</sup>.

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**Figure 1:** Selected pictures of malformed Danish piglets. A: spinal deformation, B: ear is not formed, C: cranial deformation, D: cranium hole in head, E: piglets born alive having short legs and one eye not developed, F: one large eye, an elephant trunk with bone in it, G: elephant tongue, H: female piglet with testes, I: fore gut and hind gut of the piglet with swollen belly are not connected, J: malformed piglet with swollen belly.

and feed and is able to pass the placental barriers. Further studies are warranty needed to confirm or exclude the role of glyphosate in malformations in piglets.

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