Pesticide Action Network Europe

R.I.S.K. (Rebutting Industry Science with Knowledge) Consultancy

CEO

16th July 2014

To: Mr. Tonio Borg
(cc. Mr. Url, EFSA)
European Commissioner for Health and Consumer Policy
European Commission
B-1049 Brussels.

Concerning: new evidence on the pesticide Glyphosate.

Dear Commissioner Borg,

As you will be very much aware, for many years now there has been much discussion on the adverse effects of the herbicide Glyphosate. An enormous amount of independent academic literature is published on this chemical, summarised in the Annex included in this letter. Rapporteur Germany, however, keeps on relying solely on the studies submitted by the applicants. This is a strange situation since the applicants have a huge interest in getting "the approval of the active substance". We therefore need independent studies as a counterbalance to the industry-sponsored studies.

In the co-decision procedure for the 11078/2009 pesticide regulation, all partners involved, including Commission, agreed to include the obligation to take independent peer-reviewed literature into account. In the case of Glyphosate, this obligation is not taken seriously by the applicant and Rapporteur despite more than 2000 independent studies were mentioned, all of them were dismissed for unscientific reasons, namely failing to use GLP- and the OECD Test Guidelines. Commissioner Dalli testified that GLP has no bearing on data quality, while the OECD methods cannot detect much toxicity.

We, the undersigned organization, ask you to take a look at the procedure followed by Germany and the applicants in the preparation of the Draft Assessment Report (DAR) for glyphosate; and to enforce a scientific risk assessment of glyphosate instead of a bureaucratic one.

Science published in open literature on the side effects of exposure to Glyphosate should be taken into account while placing of this active substance on the market. This is not done in the EU. Glyphosate's open literature is summarised below. Additionally, the toxicity tests

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presumably delivered by the applicants to the Rapporteur, now and in the past, should be carefully scrutinised by independent experts.

A critical re-assessment of the reliability and quality of studies delivered at the first inclusion to the Rapporteur is needed. Old analytical abilities tend to be used, controls be improper, no disease latency is allowed for, insufficient developmental toxicity is tested, and they are often 'blind' to important health endpoints. The effects elicited are from metabolism, i.e. from the quasi-poisonous doses even for chronic exposures (while effects of lower real exposures are documented only by in open literature).

In past decades several cases of fraud (IBT, Craven) were discovered by regulators and also tests on Glyphosate were among them. GLP was the answer of agencies to this test fraud. We expect those fraudulent tests were replaced by GLP-compliant ones, but still have our doubts on quality and reliability. GLP, in the end, is just a management system, forcing laboratories to write down what they do, but no-one can guarantee what is written down is exactly what happened or is observed in the tests.

Commission therefore needs to explain what guarantees they have used to ensure quality and reliability of tests, for old and new tests. Several outcomes of studies comparing GLP-studies and studies from open (independent) literature have different outcomes and this raises concern. Better to have an open comparison of the methods of these competing studies.

On top of that, Prof. Seralini of U. Caen published recently (after glyphosate's DAR was submitted to EFSA) an updated paper demonstrating that chronic (2 years) exposure of mice to Glyphosate-containing herbicide ('Roundup'), glyphosate and glyphosate-tolerant genetically modified maize (http://www.enveurope.com/content/26/1/14) resulted in kidney deficiencies, liver damage, high mortality rate, endocrine disruption and tumour development in a non-linear dose-response manner. This is the first study to examine the effects of realistic long-term exposure; previous studies of glyphosate applicants exposed only 90 days, insufficient to elicit these effects. Undoubtedly, these important realistic results should be part of the European assessment of Glyphosate, which will determine its approval and therefore whether people are protected against the risks of exposure. Not taking into account these results before approval of glyphosate means we will fail to protect people or the environment.

Since the next step in the regulatory procedure is the peer-review done by EFSA, we urge you to mandate EFSA to perform a truly independent assessment of the peer-reviewed literature, using scientifically defendable criteria of critical reviews instead of simply dismissing them for not being GLP/OECD, including the Seralini papers. The Regulation requires you to take decisions based on the most recent scientific and technological knowledge and this means experts have to start reading independent studies and decide based on the strengths and merits of the studies, not on administrative details such as GLP/OECD compliance (which is all the Klimisch guidance they use does).

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We hope you will let us know that you have sent out such a mandate to EFSA soon,

Yours sincerely,

On the behalf of the undersigned organizations,

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Annex.

Review of the scientific peer-reviewed open literature on long-term health effects of Glyphosate.

- Genotoxic effects.

Commission Review Report is quick in concluding Glyphosate(-trimesium) being "not genotoxic" ,based on a limited number of tests performed by applicants as shown in Annex A. A different picture emerges from scientific studies.

The genotoxic effects of Glyphosate were already known in open literature at the time of decision-taking of Glyphosate in 2001 and possibly not taken into account. We mention ao. the following studies:

In fruit flies, Roundup increased the frequency of sex-linked, recessive lethal mutations. These are mutations are usually visible only in males. Only a single concentration was tested in this study[1]

A study of human lymphocytes showed an increase in the frequency of sister chromatid exchanges following exposure to the lowest dose tested of Roundup[2]. Sister chromatid exchanges are exchanges of genetic material during cell division between members of a chromosome pair. They result from point mutations. A 1997 study of human lymphocytes found similar results with Roundup (at both doses tested) and with glyphosate (at all but the lowest dose tested)[3].

In *Salmonella* bacteria, Roundup was weakly mutagenic at two concentrations. In onion root cells, Roundup caused an increase in chromosome aberrations, also at two concentrations[4].

In mice injected with Roundup, the frequency of DNA adducts (the binding to genetic material of reactive molecules that lead to mutations) in the liver and kidney increased at all three doses tested[5].

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[1] Kale, P.G. et al. 1995. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ. Mol. Mutagen*. 25:148-153.
[2] Vigfusson, N.V. and E.R. Vyse. 1980. The effect of the pesticides, Dexon, Caftan and Roundup on sister-chromatid exchanges in human lymphocytes in vitro. *Mut. Res*. 79:53-57.
[3] Bolognesi, C. et al. 1997. Genotoxic activity of glyphosate and its technical formulation Roundup. *J. Agric. Food Chem.* 45:1957-1962.
[4] Rank, J. et al. 1993. Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telophase test. *Met. Res.* 300:29-36.
[5] Peluso, M. et al. 1998. ^{32}P-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environ. Molec. *Mutag*. 31:55-59.

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In another study of mice injected with glyphosate and Roundup, the frequency of chromosome damage and DNA damage increased in bone marrow, liver, and kidney. Only a single concentration was tested in this study[6].

Also after the inclusion of Glyphosate in Annex I of Directive 91/414, many studies kept on being published and showed genotoxic effects of glyphosate or the formulations of Glyphosate. We mention:

Gasnier et al.[7] studies xenobiotic effects of four different Glyphosate formulations and observed DNA damage (comet assay) at 5 ppm, as well as endocrine disrupting effects.

Manas et al. observed genotoxic effects of Glyphosate[8] and of AMPA[9]. The Comet assay in Hep-2 cells was used as well as the chromosome aberration (CA) test in human lymphocytes; potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In all three tests negative effects were found, at low doses in vitro.

Numerous other studies in test animals have demonstrated genotoxicity of Glyphosate or formulations of Glyphosate like in mouse, bovine, fish, caiman, tadpole, fruit fly, sea urchin, onion, and bacterial cells [10-11].
Also epidemiology studies showing DNA-damage in heavily sprayed areas point towards genotoxic effects of Glyphosate[12].

Given this 'weight of the evidence' it is not possible to keep to the "not genotoxic" as stated in the Review Report, and a re-assessment of the genotoxic potential of Glyphosate and its metabolite is urgently needed in the formulations used.

- Carcinogenic effects.

EU Commission's Review Report on Glyphosate (2001) concludes "no evidence of carcinogenicity" for Glyphosate (trimesium). 2-year rat studies are mentioned there but it is not sure mouse carcinogenicy studies have been performed. Considering the history of placing Glyphosate for the first time on the market in the US and considering open literature on carcinogenicity, this statement of EU Commission is hard to understand.

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[6] Bolognesi, C. et al. 1997. Genotoxic activity of glyphosate and its technical formulation Roundup. *J. Agric. Food Chem.* 45:1957-1962

[7] Gasnier, C. et al. 2009, Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines Toxicology 262: 184-191.

[8] Mañas F., Peralta L, Raviolo J, García Ovando H., Weyers A., Ugnia L., Gonzalez Cid M., Larripa I. and Gorla N., 2009a. Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. Environmental Toxicology and Pharmacology 28:37-41

[9] Mañas F., Peralta L., Raviolo J., García Ovando H., Weyers A., Ugnia L., Gonzalez Cid M, Larripa I. and Gorla N., 2009b. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety 72:834-7.

[10] Grisolia C.K., 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. Mutation Research 518:145-50.

[11] Monroy CM, Cortés AC, Sicard DM, de Restrepo HG, Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate, Biomedica. 2005 25:335-45

[12] Paz-y-Miño C., Sánchez M.E, Arévalo M., Muñoz M.J., Witte T., De-la-Carrera G.O. and Leone P.E., 2007. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Genetics and Molecular Biology 30:456-60.

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Carcinogenic effects were already known at the time of inclusion of Glyphosate in Annex I of Directive 91/414 in 2002. A previous assessment of Glyphosate in the US for instance shows the following:

The first carcinogenicity study submitted to US-EPA (1981) by applicant Monsanto showed an increase in testicular tumors in male rats as well as an increase in the frequency of a thyroid cancer in females. Both results occurred at the highest dose tested (30 mg/kg of body weight per day)[13,14]. The second study (1983) found an increasing trend in the frequency of a rare kidney tumor in male mice[15]. A later study (1990) found an increase in pancreas and liver tumors in male rats together with an increase of the same thyroid cancer found in the 1983 study in females[16].

All of these increases in tumor or cancer incidence were "not considered compound-related" [17] according to EPA. This means that EPA did not consider glyphosate the cause of the tumors. For the testicular tumors, EPA accepted the interpretation of an industry pathologist who said that the incidence in treated groups (12 percent) was similar to those observed (4.5 percent) in other rats not fed glyphosate[18]. For the thyroid cancer, EPA stated that it was not possible to distinguish between cancers and tumors of this type, so that the two should be considered together. The combined data are not statistically significant[19]. For the kidney tumors, the manufacturer re-examined the tissue and found an additional tumor in untreated mice so that statistical significance was lost. This was despite the opinion of EPA's pathologist that the lesion in question was not really a tumor[20]. For the pancreatic tumors, EPA stated that there was no dose-related trend. For the liver and thyroid tumors, EPA stated that pairwise comparisons between treated and untreated animals were not statistically significant[21]. EPA concluded that Glyphosate should be classified as Group E, "evidence of

[13] . U.S. EPA. Office of Pesticides and Toxic Substances. 1982.EPA Reg. #524-308;Lifetimefeeding study in rats with glyphosate. Memo from William Dykstra, Health Effects Division to Robert Taylor, Registration Div. Washington, D.C., Feb.18.
[14] U.S. EPA. Office of Pesticides and Toxic Substances. 1983. Glyphosate; EPA Reg. #524-308; A lifetime feeding study of glyphosate in Sprague-Dawley rats; a preliminary addendum to review dated 2/18/83. Memo to Robert Taylor, Registration Div. Washington, D.C., Feb. 15.
[15] U.S. EPA. Office of Pesticides and Toxic Substances. 1985. Glyphosate -Evaluation of kidney tumors in male mice. Chronic feeding study. Memo from L. Kassa, Toxicology Branch, to W. Dykstra, Toxicology Branch. Washington, D.C., Dec. 4.
[16] U.S. EPA. Office of Pesticides and Toxic Substances. 1991. Second peer review of glyphosate. Memo from W. Dykstra and G.Z. Ghali, Health Effects Division to R. Taylor, Registration Division, and Lois Rossi, Special Review and Reregistration Division. Washington, D.C., Oct. 30.
[17] U.S. EPA. Office of Pesticides and Toxic Substances. 1991. Second peer review of glyphosate. Memo from W. Dykstra and G.Z. Ghali, Health Effects Division to R. Taylor, Registration Division, and Lois Rossi, Special Review and Reregistration Division. Washington, D.C., Oct. 30.
[18] U.S. EPA. Office of Pesticides and Toxic Substances. 1991. Second peer review of glyphosate. Memo from W. Dykstra and G.Z. Ghali, Health Effects Division to R. Taylor, Registration Division, and Lois Rossi, Special Review and Reregistration Division. Washington, D.C., Oct. 30.
[19] U.S. EPA. Office of Pesticides and Toxic Substances. 1983. Glyphosate; EPA Reg. #524-308; A lifetime feeding study of glyphosate in Sprague-Dawley rats; a preliminary addendum to review dated 2/18/83. Memo to Robert Taylor, Registration Div. Washington, D.C., Feb. 15.
[20] U.S. EPA. Office of Pesticides and Toxic Substances. 1985. Glyphosate -Evaluation of kidney tumors in male mice. Chronic feeding study. Memo from L. Kassa, Toxicology Branch, to W. Dykstra, Toxicology Branch. Washington, D.C., Dec. 4
[21] U.S. EPA. Office of Pesticides and Toxic Substances. 1991. Second peer review of glyphosate. Memo from W. Dykstra and G.Z. Ghali, Health Effects Division to R. Taylor, Registration Division, and Lois Rossi, Special Review and Reregistration Division. Washington, D.C., Oct. 30.

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non-carcinogenicity for humans. They added that this classification "should not be interpreted as a definitive conclusion".

Regulators in the US and later in the EU gave apparently the benefit of the doubt to the applicants and used questionable interpretations to disregard tumors observed. The evidence from the scientific literature on the cancer causing potential of Glyphosate and formulations question the decision of the regulators on non-carcinogenicity.

Epidemiology studies points towards Glyphosate as a possible cause of cancer.
A number of epidemiological studies have linked exposure to glyphosate to non-Hodgkin's lymphoma[22-23] and multiple myeloma[24]. Epidemiology of course doesn't prove immediately a relation between the exposure to Glyphosate and cancer, but given the number of studies present, if should raise concern and should lead to targeted studies to find out what is going on. A further market-access of Glyphosate should be suspended as long as this additional research is ongoing.

Laboratory studies also add to the evidence of a tumor promoting potential of Glyphosate.
George et al. report tumorgenic effects of Glyphosate in mouse skin at 25 ppm per day[25].

Other ways in which glyphosate may be contributing to cancer include:
* its ability to deregulate cell division, a hall-mark of tumour cells, and demonstrated to occur in sea urchin embryos at concentrations up to 4 000 times lower than normal sprayed concentrations[26 27 28];
* its inhibition of RNA transcription, demonstrated in sea urchin embryos at concentrations 25 times lower than normal sprayed concentrations[29];
* its ability to cause oxidative stress, demonstrated for glyphosate and/or Roundup in human lymphocytes [30 31] and skin cells[32], as well as in bovine lymphocytes[33], bullfrog tadpoles[34],

[22] Nordstrom M., Hardell L, Magnuson A., Hagberg H. and Rask-Anderson A., 1998. Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. British Journal of Cancer 77:2048-52
[23] Eriksson M., Hardell L., Carlberg M. and Akerman M., 2008. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. International Journal of Cancer 123:1657-63.
[24] De Roos A.J., Blair A., Rusiecki J.A., Hoppin J.A., Svec M., Dosemeci M., Sandler D.P. and Alavanja M.C., 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *IEnvironmental Health Perspectives* 113:49-54
[25] Jasmine George, Sahdeo Prasad, Zafar Mahmood, Yogeshwer Shukla, Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach, J O U R N A L O F P R O T E O M I C S 7 3 (2 0 1 0) 9 5 1 – 9 6 4
[26] Marc J., Mulner-Lorillon O., Boulben S., Hureau D., Durand G. and Bellé R., 2002. Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chemical Research in Toxicology 15 :326-31.
[27] Marc J., Mulner-Lorillon O., Durand G. and Bellé R. 2003. Embryonic cell cycle for risk assessment of pesticides at the molecular level. Environ Chemistry Letters 1:8-12
[28] Marc J., Mulner-Lorillon O. and, Bellé R., 2004. Glyphosate-based pesticides affect cell cycle regulation. Biology of the Cell 96:245-9.
[29] Marc J., Le Breton M., Cormier P., Morales J., Belle R. and Mulner-Lorillo O., 2005. A glyphosate-based pesticide impinges on transcription. Toxicology and Applied Pharmacology 203:1-8
[30] Lioi M.B., Scarfi M.R., Santoro A., Barbieri R., Zeni O., Salvemini F., Di Berardino D., Ursini M.V. 1998. Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to glyphosate, vinclozolin, atrazine, and DPX-E9636. Environmental and Molecular Mutagenisis 32:39-46.
[31] Pieniążek D., Bukowska B. and Duda W., 2004. Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. Pesticides, Biochemistry and Physiology 79:58-63.
[32] Gehin A., Guyon C. and Nicod L., 2006. Glyphosate-induced antioxidant imbalance in HaCaT: the protective effect of vitamins C and E. Environmental Toxicology and Pharmacology 22:27-34.
[33] Lioi M.B., Scarfì M.R., Santoro A., Barbieri R., Zeni O., Di Berardino D. and Ursini M.V., 1998. Genotoxicity and

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pregnant rats and their foetuses[35], rat liver cells[36], mouse kidney cells and liver DNA[37], and in rice leaves[38].

- Reproductive toxicity and endocrine disruption

Commission Review report on Glyphosate(trimesium) of 2001 states as critical effect of reproduction "reduced pup weight at parentally toxic doses" and "litter size and pup body weight gain ↓ at parentally toxic doses" and derives a "developmental NOAEL/NOEL" of 300 mg/kg bw/d resp. 40 for trimesium. Though it is not possible on the moment to scrutinise the industry studies being at the basis of this Commission assessment, open literature shows different reproductive effects and at far lower doses. This, again, questions Commission's assessment.

Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. This reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility.

Roundup disrupted the production of steroid hormone progesterone in mouse cells by disrupting expression of a regulatory protein (StAR [39]). StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones.

Romano et al. also investigated the inhibition of StAR, this time in-vivo in Wistar rats, and their results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period[40]. They also suggest given the exposure to glyphosate in

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oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. Murationa Research 403:13-20.

[34] Costa M.J., Monteiro D.A., Oliveira-Neto A.L., Rantin F.T. and Kalinin A.L., 2008. Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. Ecotoxicolgy 173:153-63.

[35] Beuret C.J., Zirulnik F., Giménez M.S., 2005. Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. Reproductive Toxicology 19:501-4.

[36] El-Shenawy N.S., 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environmental Toxicology Pharmacology 28:39-85.

[37] Bolognesi C., Bonatti S., Degan P., Gallerani E., Peluso M., Rabboni R., Roggieri P. and Abbondandolo A., 1997. Genotoxic activity of glyphosate and its technical formulation Roundup. Journal of Agricultural and Food Chemistry 45:1957-62.

[38] Ahsan N., Lee D.G., Lee K.W., Alam I., Lee S.H., Bahk J.D., Lee B.H., 2008. Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. Plant Physiology Biochemistry 46:1062-70.

[39] Walsh L.P., McCormick C., Martin C. and Stocco D.M., 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives 108:769-76.

[40] Romano R.M., Romano M.A., Bernardi M.M., Furtado P.V. and Oliveira C.A., 2010. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology 84:309–317.

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homes of agricultural workers[41] and the exposure demonstrated by urine analysis of families living near country areas[42], may have their fertility compromised over the years.[43] [44] [45]

Richard et al. demonstrated glyphosate acts as a disruptor of mammalian cytochrome P450 aromatase activity from concentrations 100 times lower than the recommended use in agriculture; this effect is noticeable on human placental cells after only 18 hr, and it can also affect aromatase gene expression[46]. The effects are amplified by adjuvants in the formulation Roundup. The adjuvants also cause Glyphosate to be more bioaccumulative. Richard concludes Roundup to be an endocrine disrupting formulation. Moreover, at higher doses still below the classical agricultural dilutions, its toxicity on placental cells could induce some reproduction problems.
The authors suggest their research also might explain premature births and miscarriages observed in women farmers using glyphosate[47] [48].

Gasnier et al. build on this study and investigated endocrine disruption in human HepG2 liver cells. Four different endocrine disruption assays were used, two anti-estrogen (ERα and ERβ), anti-androgen (on AR) and aromatase inhibition. All tests showed endocrine disrupting properties of Glyphosate alone, but very much enhanced in a formulation (4 different formulations were tested[49]). Anti-androgen activity was already present at 0,5 ppm which is a level 800 times lower than the food standard in GM-food.

Earlier Benachour et al. demonstrated[50] that low levels of Glyphosate inhibit aromatase in human embryonic cells resulting in reduced oestrogen production, with adjuvants in Roundup increasing the effect, and with evidence of cumulative impact of doses approximating the Acceptable Daily Intake for glyphosate (0.3 mg/kg).

In a subsequent study[51] Benachour studied for the first time the mechanism of cellular action of different Roundup formulations on human cells, from placenta, embryonic kidney, and

[41] Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Warm EM, Alavanja MC (2005) Pesticide contamination inside farm and nonfarm homes. J Occup Environ Hyg 2:357–367
[42] Curwin BD, Hein MJ, Nishioka SandersonWT, MG ReynoldsSJ, Warm EM, Alavanja MC (2007) Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. J Occup Environ Hyg 51:53–55
[43] Clementi M, Tiboni GM, Causin R, Rocca CL, Maranghi F, RaVagnato F, Tenconi R (2008) Pesticides and fertility: an epidemiological study in Northeast Italy and review of the literature. Reprod Toxicol 26:13–18
[44] Foster WG, Neal MS, Han MS, Dominguez MM (2008) Environmental contaminants and human infertility: hypothesis or cause for concern? J Toxicol Environ Health 11:162–176
[45] Roeleveld N, Bretveld R (2008) The impact of pesticides on male fertility. Curr Opin Obstet Gynecol 20:229–233
[46] Richard S., Moslemi S., Sipahutar H., Benachour N. and Seralini G-E., 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environmental Health Perspectives 113: 716-20
[47] Savitz D.A., Arbuckle T., Kaczor D. and Curtis K.M., 1997. Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology 146:1025-36.
[48] Arbuckle T.E., Lin Z., Mery L.S., 2001. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Enviromental Health Perspectives 109:851-57.

[49] Céline Gasnier, Coralie Dumont, Nora Benachour, Emilie Clair, Marie-Christine Chagnon, Gilles-Eric Séralini, Glyphosate-based herbicides are toxic and endocrine disruptors in human cell Lines, Toxicology 262 (2009) 184–191
[50] Benachour N., Siphatur H., Moslemi S., Gasnier C., Travert C., Seralini G-E.. 2007. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology 53:126-33.
[51] Benachour N. and Seralini G-E., 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in Toxicology 22:97-105.

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neonate. The four Roundup herbicides and Glyphosate alone cause cellular death for all types of human cells, with comparable toxicity for each one but at different concentrations, the most toxic corresponds approximately to 47 μM Glyphosate (8 ppm) with adjuvants. Glyphosate 's toxicity begins around 1%. The mortality in all cases is not linearly linked to Glyphosate. The hypothesis that other substances are implicated has thus to be investigated in the formulation of the product. Consequently, the major Glyphosate metabolite, AMPA, and the surfactant POEA, the main claimed adjuvant by the manufacturer (the exact composition is a secret of formulation), have been tested separately from very low subagricultural dilutions (10-6 if used pure like claimed by some farmers and 10-4 if diluted as recommended at 1%). Glyphosate is claimed by the manufacturer to be the active ingredient, and it is claimed to be not toxic for human cells but toxic for vegetable ones when mixed with inert components. Benachour's study demonstrates for the first time that all products including AMPA and POEA provoke cytoplasmic membrane disruption effects in human cells, and thus mortality, but at different concentrations. The supposed inert product POEA is the most potent one. The mixture Roundup is then more poisonous than Glyphosate or AMPA. The metabolite AMPA itself destroys the cell membrane, whatever the cell type.
Paganelli et al. [52] showed in a recent study direct effect of Glyphosate formulations on early mechanisms of morphogenesis in vertebrate embryos. Paganelli showed that sublethal doses are sufficient to induce reproducible malformations in *Xenopus* and chicken embryos treated with a 1/5000 dilution of a formulant (equivalent to 430 μM of Glyphosate) or in frog embryos injected with Glyphosate alone (between 8 and 12 μM per injected cell). Formulant treated or Glyphosate injected frog embryos showed very similar phenotypes, including shortening of the trunk, cephalic reduction, microphthalmy, cyclopia, reduction of the neural crest territory at neurula stages, and craniofacial malformations at tadpole stages.

Hokanson et al.[53] also demonstrated a synergistic effect of Glyphosate with oestrogen, with implications for pregnancy-induced hypertension and foetal growth retardation.
The implications of these effects on reproduction and the developing foetus are profound, with work by Mose et al.[54] confirming that Glyphosate does cross the placenta.

- (Developmental) neurotoxicity.

Commission Review Report stating on delayed neurotoxicity "no relevant effects".
In the list of this report, any neurotoxic effects are missing. Testing is a clear data requirement but also very relevant since Glyphosate is an organophosphate and the complete class of organophosphates pesticides having neurotoxic properties. Also open literature indicates an assessment is necessary here.

[52] Alejandra Paganelli, Victoria Gnazzo, Helena Acosta, Silvia L. Lo´pez, and Andre´s E. Carrasco, Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling**,** *Chem. Res. Toxicol.* **2010,** *23*, 1586–1595
[53] Hokanson R., Fudge R., Chowdhary R. and Busbee D., 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human and Experimental Toxicology 26:747-52.

[54] Mose T., Kjaerstad M.B., Mathiesen L., Nielsen J.B., Edelfors S. and Knudsen L.E., 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. Journal ofToxicology and Environmental Health A 71:984-91.

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Epidemiology studies have been published pointing at Glyphosate of causing mental disorders of children born to pesticide applicators in the US[55]. Even acute poisoning could lead to brain damage[56].

Animal studies have demonstrated that Glyphosate depletes serotonin and dopamine[57] and causes a loss of mitochondrial transmembrane potential in rat brain cells, especially in the substantia nigra region of the brain[58].

- What exactly is the active substance?

Directive 91/414 assessed the active substances one by one and only in the national authorisations the full formulation must be assessed. It is remarkeble in the national assessements the combined effects of Glyphosate and its adjuvants haven't been considered very much by the EU member states apparently while so many scientific studies show the combined effects. In fact it would be dishonest to base an European assessment solely on tests on Glyphosate while it now appears that adjuvants are part of the active substances[59]. This is clearly a flaw in decision-making in the EU on Glyphosate. This combined effects should have assessed already in the first approval and should certainly be assessed in Regulation 1107/2009 because adjuvants need to be assessed now as well as combination effects.

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