

From: [REDACTED] (SANCO)
To: [SANCO CONSULT-E3](#)
Subject: FW: SCFCAH - glyphosate
Date: mardi 14 février 2012 10:36:56
Attachments: [Monsanto Comments on Clair et al \(Seralini\) 2012- FINAL APPROVED 1-27-12.pdf](#)
[Sanchis -Letter to the editor.pdf](#)

[pls reg and assign to DVB with FA for INFO](#)

From: [REDACTED] [mailto:[REDACTED]@monsanto.com]
Sent: Tuesday, February 14, 2012 10:09 AM
To: [REDACTED] (SANCO)
Cc: [REDACTED] (SANCO)
Subject: SCFCAH - glyphosate

Dear [REDACTED],

We note that there is a question on the agenda of the SCFCAH regarding claims about Glyphosate made in a paper by [REDACTED] 2012. We also note that there is a question from an MEP, [REDACTED], with claims about Glyphosate in groundwater in Spain which references a paper by Sanchis et al, 2011.

As in the past, we would appreciate the opportunity to provide Monsanto's comments on these papers for consideration.

- Please find attached a detailed response to the [REDACTED] paper
- Also attached is a letter to the Editor of the journal which published the [REDACTED]. In addition the water agency has said that they do not analyse for bacteria or microorganisms in the water samples because this type of groundwater is treated like surface waters and always undergoes treatment before use as drinking water. Chlorination and other water treatments remove potential traces of glyphosate.

Please do not hesitate to contact me if you need further information.

Best regards

[REDACTED]

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Monsanto Comments:

A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels

Toxicology in Vitro (on line ahead of press). Available at:
<http://www.sciencedirect.com/science/article/pii/S0887233311003341>

ABSTRACT:

The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48 h of Roundup exposure Leydig cells are damaged. Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested, and only with glyphosate in regulatory tests.

General Statement:

This publication presents no new findings relevant to the current discussions of glyphosate safety. It is clear from the previous work of [REDACTED] and others that surfactants can injure or kill cells when applied to exposed cells living in a Petri-dish environment. It also is not surprising that injured cells demonstrate activation of injury-response systems or suffer from a general decline in a wide variety of cellular functions, including hormone production in cells which normally serve that function. The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations.

The experiments reported in this publication involve two additional cell types; Leydig and Sertoli cells from rat testes. However, Petri dish experiments in a laboratory are not representative of exposures to a living animal and are not informative about real-world risks to humans. Instead, these experiments demonstrate what we already know – substances can injure unprotected cells in a test-tube. The implications of these *in vitro* experiments are contradicted by extensive live animal data, field studies reflecting real-world conditions and over 35 years of successful use of Roundup herbicide weed management around the world.

Comments:

- 1) **Glyphosate has an excellent human health and environmental profile and a long history of safe use in more than 130 countries.** This has been a key factor in the acceptance of glyphosate products as among the most widely used herbicides in the world. When used according to label directions, these products do not represent a risk to human health and the environment. This is confirmed by the extensive studies as well by the first-hand experience of millions of farmers and home gardeners who have used this product.

Glyphosate, the active ingredient in Roundup branded agricultural products, inhibits an enzyme that is essential to plant growth; this enzyme is not found in humans or other animals, contributing to the low risk to human health from the use of glyphosate according to label directions [REDACTED]. Comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (U.S. EPA, 1993 [REDACTED]); European Commission, 2002; JMPR/WHO, 2004). In fact, after a thorough review of all toxicology data available, the U.S. EPA concluded that glyphosate should be classified in Category E (“Evidence of Non-carcinogenicity in Humans”), the most favorable category possible (U.S. EPA, 1993). Glyphosate has favorable environmental characteristics, including tight binding to most soils, making it unlikely to move to groundwater or reach non-target plants, and degradation over time in soil and natural waters ([REDACTED]).

Results from these experiments are not relevant for proving toxicity in humans.

Experiments of this nature can provide useful mechanistic research tools but are not recognized or accepted by any regulatory agency or other scientific body in the world for the assessment of human health risks. The French Agency for Food Safety (AFFSA, <http://www.afssa.fr/Documents/DIVE2008sa0034.pdf>) stated that the previous work by [REDACTED] with Roundup branded formulations does not provide any elements that are relevant for proving toxicity in humans, recognizing:

- Conclusions are based on non-validated *in vitro* experiments with direct exposure to supra-physiological concentrations of substances,
- No evidence of adverse effects in regulatory studies; safety factor of 100
- No epidemiological studies allow direct incrimination,
- Direct exposure of cells can explain all the effects found in *in vitro* studies,
- Authors over interpret results for potential health consequences for humans – unsuitable references, non-sustained *in vitro-in vivo* extrapolation, etc.

- 2) **Artificial conditions.** Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism.

Anadon et al (cited by [REDACTED]) dosed rats with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 ug/ml (5mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). [REDACTED] (2012) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4% of the acceptable daily intake.

Clair et al state that the lowest concentration of glyphosate tested was 50 ppm, or 2000-fold higher than the anticipated concentration (based on [REDACTED] following maximum allowable intake. (It is further worth noting that this allowable concentration is based on a 100-fold safety factor above a no-effect level in animal studies.)

- 3) **Animal data and human experience contradicts findings of Petri dish experiments.** Glyphosate has been tested extensively in higher order animals ([REDACTED]). There is no evidence for developmental or reproductive effects in multiple species despite numerous high-dose tests by different manufacturers (Williams et al 2012, EU, 2002, JMPR/WHO 2004). Furthermore, studies with POEA have not demonstrated any target organ toxicity or effects on embryos, fetuses, or the placenta ([REDACTED]).
- 4) **The surfactant effects are not surprising.** Levine et al., 2007 demonstrated that surfactants found in household and personal care products could alter mouse Leydig cell function. It should not be a surprise that a glyphosate-based formulation which contains surfactants similar to surfactants found in household and personal care products would have an effect on cellular membranes. The exposure of humans to surfactants is common from bath gels, hand soaps, shampoos, and laundry and dishwashing detergents to name a few. In addition human oral exposure to surfactants can originate from residues on eating utensils and dishes washed with dish washing detergents and from residues taken up via drinking water (HERA, 2003).
- 5) **Caffeine metabolites, alcohol and nicotine can disrupt cell function.** It is important to note that a metabolite of caffeine inhibited the development of Leydig cells in Petri dish experiments. ([REDACTED]). In addition, alcohol ([REDACTED]) and nicotine ([REDACTED]) activate specific intracellular death-related pathways, capsase -3, inducing apoptosis in mouse Leydig cells grown in Petri dishes similar to that reported in this abstract. *In vivo* and *in vitro* exposures demonstrate that alcohol can damage Sertoli cells (Shu et al 1997). These findings clearly put this experimental model into context. Caffeine, in its natural and added forms, is found in coffee, tea, cola beverages, energy drinks, chocolate and even some medicines. The average intake of caffeine in the US by children 5-18 years of age averages 1 mg/kg/day and adults 2.4 mg/kg/day ([REDACTED]). A typical cup of coffee can contain 150 mg of caffeine, a cup of blended tea 43 mg and a small portion of a milk chocolate candy bar contains about 7 mg of caffeine. (Health Canada 2010).
- 6) **Questionable findings regarding testosterone production.** In figure-8, Leydig cell production of testosterone is reduced at a 0.0001% dilution, but is apparently normal at 5-times this concentration and remains the same (no statistical difference) up to concentrations 100-fold higher. This is biologically inexplicable and may well represent a random variation. A lack of effect on testosterone production is supported by the lack of effect on 3-beta-HSD (which is not, however, the only enzyme necessary to produce testosterone). In any event, the conclusion of the paper regarding testosterone depends upon an isolated data point inconsistent with a dose-response phenomenon, and is thus highly questionable.
- 7) **Lack of relevant new observations:** The only thing new in the experiments reported in this publication is the use of two additional cell types; Leydig and Sertoli cells from rat testes. Petri dish experiments in a laboratory are not representative of exposures to a living animal and are not informative about real-world risks to humans. Instead, these experiments demonstrate what we already know – substances can injure unprotected cells in a test-tube. The implications of these *in vitro* experiments are contradicted by extensive live animal data,

field studies reflecting real-world conditions and over 35 years of successful use of Roundup herbicide weed management around the world.

- 8) **Prior Publications.** [REDACTED] at the University of Caen in France have four prior publications on the results of exposing unprotected cells in culture to glyphosate, AMPA (aminomethylphosphonic acid, the primary environmental degradate of glyphosate), glyphosate- based formulations or a surfactant used in some formulated products.

- [REDACTED]. Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environ. Health Perspect. 113:716-720. <http://ehp03.niehs.nih.gov/article/etchArticle.action?articleURI=info:doi/10.1289/ehp.7728>
- [REDACTED]. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. Arch. Environ. Contam. Toxicol. 53:126-133. <http://www.springerlink.com/content/d13171q7k8631446/>
- [REDACTED]. Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. [REDACTED] Chem. Res. Toxicol., 22, 97–105. <http://pubs.acs.org/doi/pdf/10.1021/tx800218n>
- [REDACTED]. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology; 262(3):184-91 <http://www.sciencedirect.com/science/article/pii/S0300483X09003047>

The same group has published two publications suggesting that homeopathic remedies can protect cells against purported adverse effects of glyphosate. Co-authors are associated with the purveyor of these homeopathic products, although they claim no conflict of interest.

- [REDACTED] protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. Journal of Occupational Medicine and Toxicology 2010, 5:29 <http://www.occup-med.com/content/5/1/29>
- [REDACTED] Defined plant extracts can protect human cells against combined xenobiotic effects. Journal of Occupational Medicine and Toxicology 2011, 6:3 <http://www.occup-med.com/content/6/1/3>

- 9) **Lack of full disclosure.** The senior author on all publications is [REDACTED]. In addition to his association with the University of Caen, [REDACTED] has been the [REDACTED] of the Scientific Council for Committee for Research and Independent Information on Genetic Engineering (CRIIGEN) since 1999. [REDACTED] and the CRIIGEN organization are well known for their anti-biotechnology positions. Each of the four earlier publications state that some of the financial support for the research came from CRIIGEN. Other sources of support for his research include the “Human Earth Foundation” and “Fondation Denis Guichard”. The press releases for studies on these products can be found on the CRIIGEN website and the contact person is [REDACTED] (<http://www.crii-gen.org/>).

Additional Technical and Quality Comments:

The publication contains numerous statements and interpretations which are incorrect, and raise important questions about the scientific quality and merit of the publication. Some examples include:

- 1) Glyphosate “*penetrates and is stabilized in the cells with the help of the adjuvants* ([REDACTED])”. The cited documents are not scientific publications but “factsheets” prepared by an anti-pesticide organization. While it is clear that surfactants allow penetration into the plant by breaking down waxy cuticle substances, there is no clear evidence that the surfactants enhance cellular penetration (which is however, at least plausible) and certainly no evidence that the adjuvants in glyphosate products stabilize glyphosate in the intracellular environment.
- 2) “*G[lyphosate] and/or R[oundup] also have side targets in mammals such as cytochrome P450 reductase, StAR, aromatase and sexual steroid receptors of cells involved in reproduction or in transfected human cells* ([REDACTED] ; [REDACTED]).” The cited publications, like the current work, simply demonstrate that general cytotoxicity occurs in vitro following surfactant exposure, with concomitant decline in biosynthesis and other cellular functions. No specific targeting of these other cellular components has been demonstrated.
- 3) “*More recently it has been shown that after oral ingestion of 10 ppm of the herbicide, it diffuses in mammalian tissues, with a half-life of 15 h in rats, and G is then found in plasma at 5 ppm* ([REDACTED])”. [REDACTED] dosed glyphosate at 100 and 400 mg/kg respectively via intravenous and oral routes, not “10 ppm” as stated by [REDACTED]. The excretion half life is 9.99 hr, as opposed to the terminal half-life following ingestion, which is indeed 15 hours, but is not an excretion parameter (likely an absorption rate). As a half life is not a point in time, but rather a kinetic parameter, the statement that “*G is then found in plasma at 5 ppm*” is not rational. Peak plasma concentrations following the large oral dose did indeed range from a peak of approximately 10 ppm downwards to just below 1 ppm at 25 hours, the last time point examined.
- 4) “Moreover Leydig cells are exposed to this kind of environmental doses ([REDACTED] 2004) because 1 ppm was found in human urine and thus was present in blood. When 10 ppm of G are given to rats, half was still found in plasma 15 h later ([REDACTED] , 2009). Moreover, in testis, Leydig cells and blood vessels are in fact very close.”

The value of 1ppm appears to be an incorrect reporting of the detection limit in the [REDACTED] research. The actual detection limit was 1 part per billion. The maximal reported urinary concentration in farmers (in an individual employing poor handling practices) was 233 ppb. The highest level reported in a child, 23 ppb, occurred in a teenager who assisted in mixing and loading. The majority of farmers handling glyphosate, and the vast majority of farm spouses and children had no detectable glyphosate, i.e.- concentrations below 1 part per billion.

Equating blood and urinary concentrations displays a complete lack of basic pharmacologic understanding. Concentrations in urine may be orders of magnitude higher than blood concentrations as urinary excretion (typically 100 ml per hour) greatly exceeds plasma filtration rates (100 mg/minute). Further, all living cells are in close

proximity to vasculature- but significant barriers exist in various tissue types, including testes.

In short, the [REDACTED] offers no new findings of use. The surfactant mediated effects on cells in culture have already been documented in prior publications by this group and by others. Effects on endocrine parameters are either nil (3-beta-HSD) or highly questionable and inconsistent with dose-response relationships (testosterone). The paper is replete with technical errors and misinterpretations and adds no value to current discussions of the safety of glyphosate and GM crops..

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Letter to the editor

Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry

██████████TM (November 2011)

Dear █████,

Below some comments on the alleged glyphosate groundwater detects referred to in the publication by Sanchis *et al* (November 2011).

As the title indicates, this article evaluates the performance and accuracy of a magnetic particle immunoassay method for the determination of glyphosate in groundwater samples. Whilst the analytical part of this publication is scientifically sound and generally accurately reported, the results of the groundwater samples have been over-interpreted.

The definition of groundwater under the Water Framework Directive¹ is : *“all water which is below the surface of the ground in the saturation zone and in direct contact with the ground or subsoil”*. This means that the groundwater is under anaerobic conditions and any compound found in such groundwater must have leached through a layer of soil.

Table 1 of this publication clearly shows that, in 10 of the 11 sites, the groundwater is actually in contact with surface water. For those sites, the water cannot be associated with groundwater, as defined under the Water Framework Directive, as in this case the groundwater is not located in the saturated zone of the soil and can be contaminated by compounds present in surface water. This also means that the groundwater pesticide trigger of 0.1 µg/L does not apply.

Glyphosate presence in groundwater is rare and occurs only under exceptional circumstances. A report summarizing the glyphosate detects in European ground and surface waters (<http://www.egeis-toolbox.org/toolbox.html>) shows that less than 1% of the >36,000 analyzed groundwater samples contained glyphosate residues above 0.1 µg/L. This is in contradiction with the rate of detection of glyphosate in groundwater from this study (41%) , and also with the claim of the authors that *“very few works have been carried out to study the presence of glyphosate in groundwater”*. The same report shows that glyphosate is found in about 30% of the analyzed surface water samples, a detection frequency more in line with the observations from this publication. Based on the rate of detects from this study, and the conclusion from the author that *“the higher concentrations can be associated to sites where the sampling was carried out immediately after glyphosate application in the area”*, it seems probable that the type of sampled water is closer to surface water than to real groundwater. Additionally, the authors state that the *“sampling campaigns were carried out during the peak season of glyphosate application”* which further explains the high detection rate (even for surface water). Regarding the site 3, which does not appear to be in contact with surface water, more information would be needed to assess the vulnerability of the groundwater (depth of the groundwater, well construction, ...) and ensure that the results can be related to *“real”* groundwater. Nevertheless, given that this site is located in the same area as sites 4 and 5, it is likely that the water table from site 3 is also located in the unsaturated zone of the soil.

¹ Directive 2000/60/EC from the European Parliament and of the Council of 23 October 2000, establishing a framework for community action in the field of water policy, Official Journal of the European Communities, L327/1, 22/12/2000

In conclusion, this document provides a good scientific overview of the performance of an ultrasensitive immunoassay method for glyphosate residues in water. However, the apparent confusion on the type of sampled water doesn't allow to draw any conclusion about the glyphosate presence in groundwater.