Foetal findings	**Dose level (mg/kg bw/day)**			
	0	125	250	500
Number of foetuses (litters) with skeletal variations				
Cervical centra 1-3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extra thoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	0 (0)	3 (2)	4 (3)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

Conclusion by the Notifiers

The oral administration of glyphosate to mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and gain in body weight at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.

Comment by RMS (Re-evaluation):

The study is considered supplementary due to serious reporting deficiencies (e.g. no individual data, no statistical analysis, no uterine weights, no results of maternal necropsy). The previous NOAEL for maternal and developmental toxicity is still considered to be 250 mg/kg bw/d based on reduced food consumption and body weight gain at 500 mg/kg bw/d in does

Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: The previous evaluation did not mentioned the external malformation in rabbits which are now reported in the present RAR (abnormal tails). Total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose lever, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 78 foetuses in the high dose group (control incidence 0/109). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.

B.6.6.12 Published data (released since 2000)

A large number of studies on developmental and reproductive toxicity (DART) was published since 2000. These studies are reported and discussed below. Furthermore, also studies on endocrine disruption (ED) have been included in this chapter because they are mainly related to developmental and reproductive toxicity.

Published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include *in vitro* studies, *in vivo* studies and epidemiological studies. Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams et al. (2012, ASB2012-12052). Further discussions of significant papers follow.

In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041);
"This list should not be construed as a list of known or likely endocrine disruptors".

A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.

In Vitro EDSP Glyphosate Studies submitted to the US EPA

- Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
- Aromatase (Human Recombinant); OCSPP 890.1200
- Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
- Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455
- Published OECD Validation of the Steroidogenesis Assay (Hecker et al., 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

- Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
- *In Vivo* Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
- Female Pubertal Assay; OCSPP 890.1450; OECD None
- Male Pubertal Assay; OCSPP 890.1500
- Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
- Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic *in vivo* rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and

Uterotrophic assays. No effects were noted for any potential for glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female Pubertal assay of this programm. Based on these results, glyphosate does not exhibit endocrine disruption in Male and Female Pubertal assays.
Levine et al. (2012, ASB2014-9609) published a short summary of the results of tests with glyphosate in the EPA`s Endocrine Disruptor Screening Program (EDSP). They conclude that from the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, with a high level of confidence glyphosate would not be an endocrine disruptor.

In Vitro Glyphosate DART/ED Publications

Many *in vitro* research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using *in vitro* cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.

A laboratory at the University of Caen, France, has multiple recent publications of *in vitro* research with glyphosate and glyphosate based formulations (Richard et al., 2005, ASB2009-9024; Benachour et al., 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012-11561; Gasnier et al., 2009, ASB2012-11629; Gasnier et al., 2010, ASB2012-11628; Gasnier et al., 2011, ASB2012-11630; Clair et al., 2012, ASB2012-11592; Mesnage et al., 2012, ASB2012-11900), with proposed extrapolations to an array of *in vivo* effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the *in vitro* synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to *in vivo* effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which *in vitro* effects are reported. Again often overlooked by *in vitro* researchers and editorial boards, Levine et al. (2007, ASB2009-9030) presented convincing data demonstrating a lack of *in vitro* synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in mircosomes (Richard et al., 2005; TOX2005-1743, Benachour et al., 2007, ASB2009-9018; Gasnier et al., 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such *in vitro* systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."

Another *in vitro* publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli et al. (2010, ASB2012-11986) conducted three *in vitro*

assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a glyphosate formulation through a hole cut in the egg shell. Key issues surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang et al., (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct tranfected hormone-dependent breast cancer T47D-KBluc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity ≥ 98 %) was tested in concentrations from 10^{-12} to 10^{-6} M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these in vitro results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).
Cavalli et al. (2013, ASB2014-7495) studied the effects of the formulation Roundup Original in rat testis and Sertoli cells *in vitro*. The authors propose that Roundup toxicity, implicated in Ca^{2+} overload, cell signalling misregulation, stress response of the endoplasmatic reticulum, and/or depleted antioxidant defenses, could contribute to Sertoli cell disruption in spermatogenesis that could have an impact on male fertility.

In Vivo Glyphosate DART/ED Publications

Relatively few *in vivo* publications on glyphosate DART and ED exist in comparison with the list of *in vitro* publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, ASB2012-11601). Beuret et al. (2005, ASB2012-11564) investigated the effects of 1 % Glyphosate oral exposure (a trade product from Argentina described as "Herbicygon" was used which is a commercial herbicide formulation) on lipoperoxidation and antioxidant enzyme systems in pregnant rats and in fetuses. Lipoperoxidation was higher in both maternal and fetal livers in the glyphosate treated groups. Catalase and Superoxide dismutase activity were not altered. Both studies are reviewed in Williams et al. (2012, ASB2012-12052).

Dallegrave et al. (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are dicussed in detail in the Appendix on [redacted] (please refer to B.6.13).

Romano et al. (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce et al. (2010, ASB2012-11867). Most recently, Romano et al. (2012, ASB2012-12011) reported additional findings in male rats after supposed *in utero* and *post natal* exposures which include "behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production

and libido". As in their first publication, Romano et al. (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

Kimmel et al. (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. In summary, assessment of the reviewed data fails to support a potential risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.
Chruscielska et al. (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/day have been administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been administered.
Omran and Salama (2013, ASB2014-7614) report that the exposition of snails to atrazine or glyphosate resultet in signs of endocrine disruption and cellular toxicity. However, in this study only the formulation "Herfosate" was used and no pure active substance glyphosate.
Razi et al. (2012, ASB2014-9390) consider that glyphosate (125 mg/kg bw/d oral administered for 10, 20, 30 & 40 days) effects testicular tissue and sperm parameters in male Wistar rats. Clear effects were already seen after 10 days administration and thereafter, however accompanied by significant clinical symptoms (decreased movement, staggering gait, occasional trembling, diarrhea) and reduced body weight gain of 20 %. These findings are in contrast to those in rat studies submitted for EU evaluation. For comparison, the current EU evaluation of glyphosate proposes an overall subchronic (90-d) NOAEL of 414 mg/kg bw/d (rats) and for reproductive toxicity of 351 mg/kg bw/d, albeit generated from feeding studies. Similarily, after oral administration in female rats an NOAEL of 300 mg/kg bw/d for maternal and developmental effects was established, toxic effects were observed at much higher dose levels, only. The high toxicity described in the present publication is hardly to explain, because the publication does not give any information whether technical material or a glyphosated based formulation was tested. To conclude, the results of the publication does not affect the current assessment of glyphosate.
Cassault-Meyer et al. (2014, ASB2014-5615) investigated the effects of a glyphosate-based herbicide (Roundup Grand Travaux Plus) after an 8-day exposure of adult rats. Endocrine (aromatase, estrogen and androgen receptors, Gperl in testicular and sperm mRNAs) and testicular functions (organ weight, sperm parameters and expression of the blood-testis barrier markers) were monitored at day 68, 87, and 122 after treatment, spermiogenesis and spermatogenesis. A significant and differential expression of aromatase in testis and a diminution of mRNA expression of nuclear markers in spermatozoa were observed. The authors conclude that results suggest changes in androgen/estrogen balance and in sperm nuclear quality.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke et al. (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs (http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064809b983b). Williams et al. (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.

Furthermore, a detailed comparison of the toxicity of [redacted] and glyphosate was submitted in the appendix "Toxicological evaluation of the [redacted] surfactant (CAS no. [redacted]" which is attached to this report.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the following range of reproductive outcomes; miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry et al. (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1 % for the study population was well below the general population incidence rate of approximately 7 %. Regarding *in utero* exposures, McQueen et al. (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005 % to 2 % of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose et al., 2008, ASB2012-11914), human *in utero* exposures would be very limited.

Campana et al. (2010, ASB2013-10559) estimated the frequency of 27 birth defects in 7 geographical regions of Argentina. A sample of 21,844 newborn with birth defects was selected, ascertained from 855,220 births, between 1994 and 2007, in 59 hospitals. The study results suggested that frequencies of 14 of the 27 examined birth defects were higher in one or more regions. This study was discussed in some publications in relation to the use of glyphosate pesticides. However, Campana et al. (2010, ASB2013-10559) commented on secular trends, altitude above sea level, folic acid fortification and ethnic factors and further variables. It was not indicated that any of these variables was associated with an increased occurrence of any type of birth defects.

Two studies of residential proximity to agriculture-related pesticide applications (California) by Carmichael et al. (2013, ASB2014-9307) and Yang et al. (2013, ASB2014-9644) examined whether early gestational exposure to pesticides were associated with an increased risk of hypospadia, neural tube defects or orofacial clefts in offspring. In both studies formulated glyphosate was mentioned only as one out of five chemicals to which controls were most frequently exposed. The authors of both studies concluded the few positive findings on chemicals, but other than glyphosate, should be interpreted with caution and need to be repeated in other populations.

Manfo et al. (2010, ASB2014-9611) examined the effect of pesticides use on male reproductive function in a study on farmers in Cameroon. The farmers of Djutitsa (West Cameroon) used 25 active substances (in 57 preparations) amongst others glyphosate in different formulations and were exposed to agro-pesticides due to inappropriate handling and improper protective tools. Furthermore, the authors concluded, that male farmers, who are exposed to pesticides might have impaired reproductive function through inhibition of testosterone synthesis. Serum biochemical parameter (total testosterone, estradiol/testosterone, androstenedione) were altered compared to the unexposed control group, but these alterations of chemical parameter cannot be related to single pesticides, *e.g.* glyphosate. Moreover, the fungicides were the most used active ingredients. However, considering the obvious alterations, the authors concluded, that there is urgent need for more

training to enable improvement of equipment and efficiency of application to minimize exposure risks.

Further reviews on DART

Antoniou et al. (2012, ASB2012-15927) submitted a review article on "Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence". According to the authors published studies "have raised concern regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems". The "draft assessment report revealed that … industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity". The EU adopted "an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans." The authors suggest that a "new risk assessment should be conducted with full public transparency by scientists who are independent of industry."

Lopez et al. (2012, ASB2013-10534) submitted a review article on "Pesticides used in South American GMO-Based Agriculture: a review on their effects on humans and animal models". The authors discuss the results of genetic studies in agricultural regions in the province of Cordoba, Argentina, biomarkers in agricultural regions in the province of Santa Fe, Argentina and congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay. According to the authors, human health in these areas was damaged by pesticides. However, a relation to glyphosate or another substance or pesticide was not evidenced. Nevertheless, based on the results of Paganelli et al. (2010, ASB2012-11986), it was concluded that glyphosate-based herbicides) would be linked to an increased activity of the retinoic acid signaling pathways and this might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

Basrur (2006, ASB2014-7492) submitted a review on disrupted sex differentiation and feminization of men. In this review the studies of Arbuckle and associates are cited which report a relation between pesticide exposure (including glyphosate) and reproductive risk.

Vandenberg et al. (2012, ASB2014-9635) submitted a review on low dose effects an nonmonotonic dose responses of hormones and endocrine disrupting chemicals. The authors reviewed two major concepts on EDC studies: low dose and nonmonotonicity. They conclude that nonmonotonic responses and low-dose effects would be remarkably common in studies of natural hormones and EDSs. Whether low doses of EDCs influence certain human disorders would be no longer conjecture, because epidemiologic studies would show that environmental exposures to ECDs would be associated with human diseases and disabilities. The authors demand that fundamental changes in chemical testing and safety determination woud be needed to protect human health.

In a direct response on the article of Vandenberg et al. (2012, ASB2014-9635) a discussion paper was submitted by Rhomberg and Goodman (2012, ASB2014-9391). These authors conclude that Vandenberg et al. (2012, ASB2014-9635) presented examples as anecdotes without attempting to review all available pertinent data, selectively citing studies without evaluating most of them or examining whether their putative examples are consistent and coherent with other relevant information. Many of their examples have been questioned by many scientists. Overall, Vandenberg et al. (2012, ASB2014-9635) put forth many asserted illustrations of their two conclusions without providing sufficient evidence to make the case for either and while overlooking evidence that suggest the contrary.

Lamb et al. (2014, ASB2014-9605) submitted a review with critical comments on the WHO-UNEP state of the science of endocrine disrupting chemicals – 2012. The authors conclude

that the 2012 report does not provide a balanced perspective, nor does it accurately reflect the state fo the science on endocrine disruption.
Borgert et al. (2013, ASB2014-9292) reviewed literature on thresholds of endocrine activity. The brief review highlights how the fundamental principles governing hormonal effects – affinity, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.
The review by Sengupta and Banerjee, (2013, ASB2014-9730) is related to impacts of pesticides on male fertility. With respect to glyphosate the authors only cited in vitro data published by Richard et al. (2005, ASB2009-9025), and these have been already reported and evaluated in the present renewal assessment report (please refer to '*In vitro* Glyphosate DART/ED Publications').
Kumar (2011, ASB2014-9725) submitted a review on occupational, environmental and lifestyle factors associated with spontaneous abortion. In this review Arbuckle et al. (2001, ASB2012-11545) was cited who reported a relation between pesticide exposure (including glyphosate) and reproductive risk. This publication was already reported and discussed under 'Epidemiology DART/ED Publication'.
The extensive review by Wigle et al. (2008, ASB2014-9637) summarised the level of epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. Several references related to glyphosate were cited by the authors [(Curtis et al.1999, cited in Arbuckle et al. (2001, ASB2012-11545), Arbuckle et al. (2001, ASB2012-11545), Savitz et al. (1997, ASB2012-12022), Garry (2002, ASB2012-11626))], which were already reported and discussed under to 'Epidemiology DART/ED Publication'.
The mechanism based short review by Jamkhande et al. (2014, ASB2014-9573) summarised common human teratogenic agents. With respect to glyphosate (-based formulations) the authors cited merely data published by Antoniou et al. (2012, ASB2012-15927); Paganelli et al. (2010, ASB2012-11986). Both publications were already reported and evaluated in the present renewal assessment report (please refer to 'Further reviews on DART').
The English abstract of a Chinese publication by Zhang et al. (2013, ASB2014-9643) give notice of a summary on reproductive and developmental toxicity studies on glyphosate and the related mechanisms on humans and animals to provide suggestions for further research.

Comparison of the active substance glyphosate and glyphosate containing formulations concerning DART and ED

For the active substance glyphosate a very comprehensive data package of guideline conform studies on developmental and reproductive toxicity is available. This data package was prepared over the last decades and updated within the last years.
In these submitted studies it was demonstrated that glyphosate is not a teratogenic substance. NOEL values for developmental toxicity and reproductive toxicity can be derived from the results of these studies. There are no relevant indications of an endocrine disrupting activity of the active substance glyphosate. Additionally, also in the further guideline conform toxicological studies (e.g. the subchronic and chronic toxicity studies) no indications of an endocrine disrupting activity of glyphosate (e.g. organ weight and histology of sexual organs, behaviour etc.) have been observed. Therefore, on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance.
Additionally to the studies which have been performed according to validated EU- and OECD guidelines a large number of studies has been published on DART and ED. Most of these

studies use glyphosate containing preparations instead of the pure active substance glyphosate. However, some studies directly compare the toxicity of the active substance glyphosate and glyphosate conaining preparations. Furthermore, studies have been performed on the toxicity of surfactants which are used in preparations together with glyphosate, especially [redacted] The results of these surfactant studies can be compared with the results of the above mentioned guideline conform studies on glyphosate.

In result of these comparisons it can clearly be concluded that the toxicity of preparations and the toxicity of surfactants like [redacted] / polyethoxylated alkylamine is significantly higher than the toxicity of the active substance glyphosate.

A detailed comparison of the toxicity of tallowamin and glyphosate was submitted in the appendix "Toxicological evaluation of the [redacted]e surfactant (CAS no. 61791-26-2)" which is attached to this report. In this evaluation is clearly demonstrated that there is a significantly higher toxicity of the surfactant tallowamin with regard to all of the following endpoints investigated:
- acute oral toxicity
- acute dermal toxicity
- skin irritation
- eye irritation
- skin sensitization
- short term toxicity, rat
- short term toxicity, dog
- reproduction toxicity study, parental toxicity
- reproduction toxicity study, reproductive toxicity
- reproduction toxicity study, offspring toxicity
- developmental toxicity, rat, fetal effects

Walsh et al. (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine et al. (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.

Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009, ASB2012-12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs et al. (2012, ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells *in vitro*. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker et al., 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and

Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Richard et al. (2005, TOX2005-1743) studied effects of glyphosate and roundup on human placental cells and aromatase. Summarising their results they stated that "roundup is always more toxic than its active ingredient."
In a further study from the same institute Benachour et al. (2007, ASB2009-9018) studied time- and dose-dependent effects of roundup on human embryonic and placental cells. They summarized that "in all instances, roundup … is more efficient than its active ingredient, glyphosate…". And in a further publication by Benachour and Seralini (2009, ASB2012-11561) it was stated "this work clearly confirms that the adjuvants in roundup formulations are not inert." In a response to this publication by the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) it was answered that surfactant effects … are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis. In the most recent publication from the same institute, Mesnage et al. (in press, ASB2012-13917) the potential active principle for toxicity on human cells for 9 glyphosate-based formulations was studied. The authors summarized that "ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity".

In a comprehensive analysis of the available literature in developmenat and reproductive outcomes in humans and animals after glyphosate exposure, Williams et al. (2012, ASB2012-12052) summarized: "An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanism of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure."

***In vitro* DART/ED publications**

Author(s)	**Year**	**Study title**
Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives Volume: 108 Number: 8 Pages: 769-776 ASB2012-12046

Abstract*
Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide

Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. 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. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3 β-hydroxysteroid dehydrogenase (3 β-HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl [$(Bu)_2$]cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.
*Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in Roundup confounds results.
Relevance of study:	Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the observed effects are result of glyphosate exposure.
Klimisch code:	2

Additional comments:
Glyphosate did not affect steroidogenesis in the test system.
Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.
Roundup results were comprehensively addressed in Levine et al. (2007, ASB2009-9030):
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have “indistinguishable” dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.
Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.
The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.
StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh et al. (2000, ASB2012-12046).

Author(s)	Year	Study title
Paganelli, A. Gnazzo, V. Acosta H. Lopez, S.L. Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling Chemical Research in Toxicology Volume: 23 Pages: 1586-1595 ASB2010-11410

Abstract*
The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:	Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)

No pH adjustment for doses and thus effects may be in response to the acidic nature of glyphosate technical acid.
Inappropriate and irrelevant routes of exposure.
Data requires further substantiation before consideration in risk assessment.

Response 2 – Saltmiras et al. (2011, ASB2012-12015) letter to the Editor
Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant.
The authors' justification for this research is flawed, providing no valid basis, other than an opinion, of an increase in the rate of birth defects in Argentina.
Direct injection of frog embryos and through chicken shells do not reflect real world exposure scenarios to either environmental species or humans.
Doses were excessively high and irrelevant for risk assessment purposes. Frog embryos were also bathed in glyphosate formulation at doses 9-15 times greater than the acute LC50 same species of frog. Calculating equivalent oral doses based on pharmacokinetics studies, such doses are 150000000 times greater than worst case human exposure monitoring data.
"…. the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies."

Response 3 – Mulet (2011, ASB2012-11916) letter to the Editor
Notes the premise for this research is falsely based on an incorrectly cited local pediatric bulletin from Paraguay.
"…. this article refers to a study in a single hospital in Paraguay showing a correlation between pesticide use (not herbicides as mentioned by Paganelli et al., ASB2010-11410) and birth malformations. In the cited study (Benitez et al., ASB2012-11563), the authors state that the results are preliminary and must be confirmed. Is important to remark that the Benitez et al. study does not include any mention to glyphosate, so does not account for what the authors are stating in the introduction…..This journal is also wrongly cited in the discussion referring to increased malformations due to herbicides, which is not the result of the study."

Response 4 – comments from BVL (2010, ASB2012-11579)
Highly artificial experimental conditions.
Inappropriate models to replace validated mammalian reproductive and developmental toxicity testing methods for use in human health risk assessment.
Inappropriate routes of exposure.
Lack of corroborative evidence in humans.
"In spite of long-lasting use of glyphosate-based herbicides worldwide, no evidence of teratogenicity in humans has been obtained so far."

Response 5– comments from European Commission Standing Committee on the Food Chain and Animal Health (2011, ASB2012-11615)
The EU commission supports the German Authorities position, "that that there is a comprehensive and reliable toxicological database for glyphosate and the effects observed have not been revealed in mammalian studies, nor evidenced epidemiologically in humans."
"…. the Commission does not consider there is currently a solid basis to ban or impose specific restrictions on the use of glyphosate in the EU."

Response 6– Palma, G. (2010, ASB2012-11989) letter to the Editor

The author of the letter claims that the study by Paganelli et al., 2010 (ASB2010-11410), described effects of glyphosate only at unrealistic high concentrations or via unrealistic routes of exposure. The data are thought to be inconsistent with the literature, and therefore not suitable or relevant for the risk assessment for humans and wildlife. Furthermore the author asserts that findings do not support the extrapolation to human health as stated in the publication.

Author(s)	**Year**	**Study title**
Richard, S. Moslemi, S. Sipahutar, H. Benachour, N. Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives Volume: 113 Pages: 716-720 TOX2005-1743

Abstract*
Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all glassware should be thoroughly rinsed.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate at non-cytotoxic concentrations in this test system was demonstrated to have no effects on aromatase activity.
Likewise, did not affect mRNA levels after 18 hours treatment at ≤ 0.1% glyphosate.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
The *in vitro* test system is non-validated
Physiologically irrelevant concentrations tested.
Testing surfactant-like substances in such systems is now recognized to be not valid.

Response 2 – summarized from the French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005, ASB2009-9025)
Major methodological gaps.
JEG3 cells, a choriocarcinoma human cell line (average of 70 chromosomes vs 46 in normal human cells).
Concentrations of Roundup used in the various experiments considered to be extremely high.
In consideration of limiting factors (oral absorption, 30 %; skin absorption, 0.3 %; rapid elimination kinetics), such levels would involve considerable human exposure, or several dozen liters of Roundup diluted at 2 %.
concentrations of Roundup that trigger an effect on aromatase (0.5 % - 2 %) are at least 1000 times more effective than those of known aromatase inhibitors, such as azole derivatives
Study design does not make it possible to show the influence of the adjuvants, nor synergism of adjuvants and glyphosate.
Multiple non-specific effects of surfactant agents on a broad range of cellular targets not discussed.
No comparison with comparable surfactant agents intended for household use.
Multiple instances of bias in its arguments and its interpretation of the data.
The authors over-interpret their results in the area of potential health consequences for humans (unsuitable references, non-sustained in vitro-in vivo extrapolation, etc.).

Author(s)	Year	Study title
Benachour, N. Sipahutar, H. Moslerni, 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 Volume: 53 Pages: 126-133 ASB2009-9018

Abstract*
Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD_{50}) of Roundup with embryonic cells is 0.3 % within 1 h in serum-free medium, and it decreases to reach 0.06 % (containing among other compounds 1.27 mM glyphosate) after 72

h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1 -2 %, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01 % (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:
Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.
Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.
Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker et al., 2011, ASB2012-11840).

Response – summarized from Williams et al. (2012, ASB2012-12052)

pH of test system not adjusted to physiologically appropriate levels;
Negative controls were not pH adjusted to appropriate levels.
Confounding surfactant effects due to cell membrane damage render data generated with formulated products in this test system null.

Author(s)	Year	Study title
Benachour, N. Seralini, G. E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in toxicology Volume: 22, Pages: 97-105 ASB2012-11561

Abstract*
We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from formulation-treated crops.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. No positive controls were included.
Relevance of study:	Not relevant (Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with

surfactants)

Klimisch code: 3

Additional comments:

Response – summarized from the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532)
Cell lines used present characteristics which may be at the source of a significant bias in the interpretation of the results.
Experiments were conducted with 24 hours exposure in a medium without serum, which could lead to disturbance of the physiological state of the cells.
The glyphosate used in the study is glyphosate acid, whereas in the preparations tested it is in the form of an isopropylamine salt. No precise information is given about the pH of test concentrations except the highest dose.
No mention of any positive evidence for the apoptosis test.
Cytoxicity and induction of apoptosis may due to pH and/or variations in osmotic pressure on cell survival at the high doses tested.
Surfactant (tensoactive) effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.
Conclusions are based on unvalidated, non-representative cell models (in particular tumour or transformed cell lines) directly exposed to extremely high product concentrations in culture conditions which do not observe normal cell physiological conditions.
No new information is presented on mechanism of action of glyphosate and preparations containing glyphosate.
The authors over-interpret their results with regard to potential health consequences for humans, based in particular on an unsupported *in vitro–in vivo* extrapolation
The cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations proposed by Benachour and Seralini do not add any pertinent new facts which call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations.

Author(s)	**Year**	**Study title**
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology Volume: 262, Number: 3, Pages: 184-191 ASB2012-11629

Abstract*
Glyphosate-based herbicides are the most widely used across the world; they are commercialised in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERα, ERβ) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters

were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Due to reporting deficiencies (e.g. correlation between concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate demonstrated no significant anti-estrogenic potential
Glyphosate demonstrated some anti-androgenic potential at lower concentrations, but not as doses increased and therefore results are considered unrelated to treatment
Four glyphosate based formulations demonstrated both estrogenic and androgenic activity.
Results are confounded due to surfactants within the formulated products tested, which affect cell membrane integrity and produces false findings.

Response 2 – summarized from BfR Review (2009, ASB2012-11565)
Numerous methodological flaws are noted.
Test substance(s) not characterized
Source of materials for cell culture not provided.
Dosing concentrations not well described
Serum free media only appropriate for short term (3-4 hour) *in vitro* exposures.
pH control of dilutions not clear.
Osmolality of test solutions not reported.
Electrophoresis parameters insufficiently or inaccurately reported.
Numerous reporting deficiencies are noted.
Influence of serum-free cell culturing on endpoints can not be determined
Incomplete data reporting, in cluding β-galactosirase activity, cototoxicity for select assays.
Positive control data not reported.

Confusion between maximum residue levels verses systemic concentrations in humans.

Author(s)	Year	Study title
Clair, E., Mesnage, R., Travert, C., Seralini, G.E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. Toxicology in Vitro Volume: 26, Number: 2, Pages: 269-279 ASB2012-1628

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.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline *in vitro* test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).
Relevance of study:	Not relevant (Due to reliability. In addition, *in vitro* data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.)
Klimisch code:	3

Additional comments:
In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). 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. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on

pharmacokinetics described in Anadon et al., 2009, ASB2012-11542) following worst case dietary exposure at the ADI.

Author(s)	Year	Study title
Hokanson, R. Fudge, R. Chowdhary, R. Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology Volume: 26, Pages: 747-752, ASB2012-11846

Abstract*
Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized *in vitro* DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed *in utero*.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Not acceptable *in vitro* methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test substance in cell lines.
Relevance of study:	Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. *In vitro* cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:	3

Additional comments:
In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.

In vivo DART/ED publications

Author(s)	Year	Study title
Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K.	1995	Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits. Journal of Environmental Science and Health. Part B. Volume: 30, Number: 4, Pages: 513-534 ASB2012-12058

Abstract*
The present study was undertaken to investigate the effect of chronic treatment with two sublethal doses of Carbofuran (carbamate insecticide) and Glyphosate (organophosphorus herbicide) on body weight and semen characteristics in mature male New Zealand white rabbits. Pesticide treatment resulted in a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. The hazardous effect of these pesticides on semen quality continued during the recovery period, and was dose-dependent. These effects on sperm quality may be due to the direct cytotoxic effects of these pesticides on spermatogenesis and/or indirectly via hypothalami-pituitary-testis axis which control the reproductive efficiency.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD_{50}. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits have low body weights at study start, suggesting impaired health status.
Relevance of study:	Not relevant (Due to low confidence in study conduct and the inadequacy of reporting.)
Klimisch code:	3

Additional comments:

Response – summarized from Williams et al. (2000, ASB2012-12053)
Numerous serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable.
Only four rabbits per treatment group were used, and therefore statistics are questionable.

Rabbits appeared to be small for their age; at study start (32 weeks) tested animals had 16-25 % lower body weight than historical weights for commercially bred animals of the same age and strain.
Low body weights as study start suggest compromised health status of the animals at initiation.
Dose levels were not quantified.
Purity of glyphosate and composition of the glyphosate formulation were not reported.
Inadequate description of test material administration.
Improper semen collection technique reported.
Report is unclear whether control animal sham handling was undertaken, a critical factor in stress related outcomes in this species.
Food consumption of test and control groups not adequately reported.
Variability not adequately reported for endpoint measurements in test and control groups, preventing statistical analysis to support the author's conclusions.
Dose-responses not observed, despite the wide dose spread.
Sperm concentrations of all groups within normal ranges for this strain of rabbit.
No meaningful conclusions can be drawn from this publication.

Author(s)	**Year**	**Study title**
Daruich, J. Zirulnik, F. Gimenez, M. S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research Volume: 85 Pages: 226-231 ASB2012-11601

Abstract*

To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured, inappropriate controls, lack of consistent dose-response data).

Relevance of study:	Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code:	3

Additional comments:
The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.

Response summarized from Williams et al. (2012, ASB2012-12052)
Test substance and doses not adequately described.
Inappropriate control groups.
Results suggest that the effect of treatment on body and organ weights may be due to reduced food and water intakes.
A consistent effect of treatment was not observed and dose-response relationships were generally lacking
The information gathered may be misleading because the enzymes monitored are found in both the cytosol and mitochondria.
Food restriction affects the activity of many enzymes, including those examined in this study.

Author(s)	Year	Study title
Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology Volume: 84, Pages: 309-317 ASB2012-12012

Abstract*
Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250

mg/kg = 76.9 ± 14.2 ng/dL). These 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.
Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular weights, but decreased testosterone measurements.
Relevance of study:	Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test material was a formulated product, not glyphosate).
Klimisch code:	3

Additional comments:
Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.

Author(s)	**Year**	**Study title**
Romano, M.A. Romano, R.M. Santos, L.D. Wisniewski, P. Campos, D.A. de Souza, P.B. Viau, P. Bernardi, M.M. Nunes, M.T. de Oliviera, C.A.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology Volume: 86, Number: 4, Pages: 663-673 ASB2012-12011

Abstract*
Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the

serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. *In vivo* study with reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A number of atypical endpoints evaluated.
Relevance of study:	Not relevant (due to questionable dosing regimen and atypical array of endpoints measured).
Klimisch code:	3

Additional comments:
Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as "sexual partner preference" along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano et al. (2010, ASB2012-12012) compared to Romano et al. (2012, ASB2012-12011); these include increased day of preputional sepratarion (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 µm in 2010; 479 µm in 2012), epithelial height (86 µm in 2010; 92 µmin 2012) and luminal height (94 µm in 2010; 257µm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.

A letter to the editor by DeSesso and Williams, (2012, ASB2014-9369) concluded as follows: "Taken together, the shortcomings in this paper erode any confidence that these experiments are able to demonstrate disruption in the development or function of the male reproductive

system in offspring whose dame were treated with glyphosate". Romano and Romano (2012, ASB2014-9396) rebutted these comments and conclusions.

Epidemiology DART/ED Publications

Author(s)	Year	Study title
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 Environmental Health Perspectives Volume: 109 Pages: 851-857 ASB2012-11545

Abstract*

The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide.
Relevance of study:	Not relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:

Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated

with spontantous abortin (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- or/and post-conception
OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60 % accuracy when comparing self reported pesticide usage with purchasing records.
OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.
Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.

Response summarized from Williams et al. (2012, ASB2012-12052)
395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001, ASB2012-11545) was 10 %.
The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 12 % to 25 %.
Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34% of all spontaneous abortions reported).
Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.
Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.
Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.

Author(s)	Year	Study title
Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036 ASB2012-12022

Abstract*
Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-for-gestational-age births. Among the 1,898 couples with complete data (64 % response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95 % confidence interval 1.0-4.4), Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.
Relevance of study:	Not Relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:

Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz et *al.* (1997, ASB2012-12022), Curtis et *al.* (1999, cited in ASB2012-11545) and Arbuckle et *al.* (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz *et al.* (1997, ASB2012-12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth. Curtis *et al.* (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle *et al.* (2001, ASB2012-11545) looked for associations between reported pesticide use and spontaneous abortion.

In the study by Savitz et al. (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis et al.(1999, cited in ASB2012-11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle *et al.* (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies.
These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:

There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing records (Blair & Zahm 1993, ASB2012-11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were

assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.

The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.

The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes that are not validated with medical records.

A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.

In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz *et al.* 1997, ASB2012-12022, Curtis *et al.* 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.

Biomonitoring data for glyphosate, collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel *et al.*, 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 *mg/kg* (Acquavella *et al.*, 2004, ASB2012-11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose1 of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.

Author(s)	Year	Study title
Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental Health Perspectives Volume: 110 Pages: 441-449 ASB2012-11626

Abstract*

We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent-reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5. 1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that a) herbicides applied in the spring may be a factor in the birth defects observed and b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).
Relevance of study:	Not relevant because of methodological deficiencies.
Klimisch code:	3

Additional comments:

Response 1 – summary from Mink et al. (2011) (ASB2012-11904)
Publication reports on different classes of pesticides and several birth defects and developmental outcomes.
Paternal use of glyphosate was associated with parent-reported ADD/ADHD in children (OR = 3.6). Six out of 14 children with parent reported ADD/ADHD also reported exposure to glyphosate.
Diagnoses of ADD/AHDH were not all confirmed. However, overall rate for the sample population (14/1532) was well below ADD/ADHD rates for the general population (7%).
Variables in statistical model analyses were not reported.

Response 2 – summary from Williams et al. (2012, ASB2012-12052)
Health data obtained via parent reporting for 695 families via written questionnaire and confirmed where possible.
Pesticide use information obtained initially via telephone then followed up by written questionnaire.
Reproductive health outcomes for births occurring between 1968 and 1998 were obtained for 1532 live births. Over half the births occurred prior to 1978, approximately 20 years after study initiation.
All pesticide use classes (herbicide only; herbicide and insecticide; herbicide, insecticide and fungicide; herbicide, insecticide and fumigant) were associated with birth defects.
Authors state neurobehavioral disorder would not be considered based lack consistent diagnoses. However, a detailed analysis was conducted for ADD/ADHD.
43% (6/14) parent reported children with ADD/ADHD were associated with glyphosate formulation use.
14 cases of ADD/ADHD reported out of 1532 live births, which is substantially lower that the diagnosed incidence of 7% for the general population.
No conclusions regarding glyphosate exposure and ADD/ADHD outcome can be drawn.
No other glyphosate specific data were reported.

Author(s)	Year	Study title
Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male Reproductive Hormones and Thyroid Function in Pesticide Applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health, Part A Volume: 66, Number: 11, Pages: 965-986 ASB2012-11627

Abstract*
In the present effort, 144 pesticide applicators and 49 urban control subjects who reported no chronic disease were studied. Applicators provided records of the season's pesticides used by product, volumes, dates, and methods of application. Blood specimens for examination of hormone levels were obtained in summer and fall. In the herbicide-only applicator group, significant increases in testosterone levels in fall compared to summer and also elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the fall were noted. With respect to fungicide use, in an earlier cross-sectional epidemiologic study, data demonstrated that historic fungicide use was associated with a significant alteration of the sex ratio of children borne to applicators. As before, among current study subjects it was noted

that historic fungicide use was associated with increased numbers of girls being born. Lower mean total testosterone concentrations by quartile were also correlated with increased numbers of live-born female infants. A downward summer to fall seasonal shift in thyroid-stimulating hormone (TSH) concentrations occurred among applicators but not among controls. Farmers who had aerial application of fungicides to their land in the current season showed a significant shift in TSH values (from 1.75 to 1.11 mU/L). Subclinical hypothyroidism was noted in 5/144 applicators (TSH values >4.5 mU/L), but not in urban control subjects. Based on current and past studies, it was concluded that, in addition to pesticide exposure, individual susceptibility and perhaps economic factors may play a supporting role in the reported results.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure). Documentation is insufficient for assessment.
Relevance of study:	Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure was made).
Klimisch code:	3

Additional comments:
The publication brings little information on endpoints attributable to glyphosate.
Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances.
Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.
Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.

Author(s)	Year	Study title
Bell, E.M. Hertz-Picciotto, I. Beaumont, J.J.	2001	A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies Epidemiology Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559

Abstract*
We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and

county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd– 8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95 % confidence interval = 0.8 – 2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95 % confidence interval = 1.3 – 3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd– 8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemoliogical study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).
Relevance of study:	Not relevant (No glyphosate-specific results.)
Klimisch code:	3

Additional comments:

Response – summary from Williams et al. (2012, ASB2012-12052)
Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of "phosphates/thiophosphates/phosphonates".
Of the 47 active ingredients, many were organophosphate insecticide with known mammalian modes of action. The glyphosate mode of action is on the EPSPS enzyme in plants, which is not present in the animal kingdom.
Given the very low volatility of glyphosate and the low potential for inhalation exposures to aerosol sprays up to two miles away from the subjects, systemic doses to glyphosate would be considered negligible.
Mose et al., (2008, ASB2012-11914) demonstrated a low perfusion rate of glyphosate across the placenta. Coupled with the known low dermal and gastrointestinal absorption of glyphosate and the rapid elimination of systemic doses of glyphosate in the urine, human *in utero* exposures would be extremely limited.
The reported congenital anomalies associated with fetal death in Bell et al. (2001, ASB2012-11559) can in no way be linked to glyphosate exposure.

Author(s)	Year	Study title
Aris, A. Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547

Abstract*

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other exposure routes.
Relevance of study:	Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied pesticides, application rates, etc.).
Klimisch code:	3

Author(s)	Year	Study title
Benítez-Leite, S. Macchi, ML and Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos. Arch Pediatr Urug Volume: 80, Number: 3, Pages: 237-247 ASB2012-11563

Abstract*
Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn. Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnación, in the Department of Itapúa, Paraguay. Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects. Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, p<0,02), dwelling located less than 1 km (OR 2,66, CI95% 1,19-5,97, p<0,008), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63), p<0,003), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, p<0,04), and family history of malformation (OR 6,81, CI95% 1,94-30,56, p<0,001). Other known risk

factors for malformations did not show statistical significance. Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).
Relevance of study:	Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance, including glyphosate, was specified or assessed).
Klimisch code:	3