Table 47. Summary of information supporting EDSP data in relation to glyphosate and endocrine end-points

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Estrogen pathway						
EDSP Tier 1 data 2014/2015: In vitro: ER binding; TG 455 ER STTA and Hela Assay In vivo: mammalian assays, i.e. uterotrophic and female pubertal assays and mammalian toxicity studies	Glyphosate Purity: 85.1–95.93% Concentration range: 10^{-10} to 10^{-3} mol/L	USEPA validated assays In vitro assays are well-characterized and OECD TGs ER STTA: uses HeLa cell line which has ER α not ER β. ER α perturbation is more strongly associated with adverse outcomes	There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies, however decreases in offspring body weight were observed in one avian reproduction study	High	Negative	USEPA (2015)
In vitro: ER agonism in estrogen-dependent T47D human breast cancer cells	Glyphosate Purity > 98%) Accustandard Concentration range: 10^{-12} to 10^{-6} mol/L	Validated assay	Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, and not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} mol/L in estrogen withdrawal condition, which was reported to be confirmed by the inhibitory effect of the ER antagonist ICI 182780. The T47D cell line contains both ERα and ERβ. While the use of ICI 182780 can exclude the possibility of dioxin-like interference of coformulant contaminant 1,4-dioxane with AhR interactions affecting the ER, this study is confounded because it was not tested with an ER*α*-specific antagonist, such as methylpiperidino pyrazole (CAS No. 289726-02-9). This would determine the relative activities of each ER (Evans, Gray & Wilson, 2012) The luciferase reporter system was then also used with combinations of genistein, an isoflavone in soy. Phytoestrogens such as genistein are known to overstimulate luciferase, and also are stronger ligands for ERβ. Non-receptor–mediated luminescence signals have	Low	Positive	Thongprakaisang et al. (2013)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			been reported at phytoestrogen concentrations higher than 1 µmol/L due to the over-activation of the luciferase reporter gene (Kuiper et al., 1998; Escande et al., 2006). While the dose–response curve indicates that true activation of the ER system occurs at lower concentrations, luciferase expression obtained at high concentrations of phytoestrogens or similar compounds suspected of producing phytoestrogen-like over-activation of the luciferase reporter gene needs to be examined carefully in stably transfected ERTA assay systems. (See Annex 2 of OECD TG 455)			
In vitro: hERα and hERβ (ant)agonism in reporter gene–transfected CHO cells	Glyphosate (> 95–100%); whether this is a formulation is not specified in the paper. Concentrations for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.		Concentrations of pesticides that tested negative, which included glyphosate, are not reported; only the results of those that tested positive are provided. Concentration of positively testing chemicals ranged from 10^{-6} to 10^{-12} mol/L Concentration of the test chemicals showing 20% of the agonistic activity of 10^{-10} mol/L 17β-estradiol, and is given as REC 20(mol/L)	Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hERα and hERβ transient transfection into human hepatocarcinoma HepG2 cells	Glyphosate formulations and glyphosate parent chemical. Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line	Formulations reduced transcription of *ERα* and *ERβ* in HepG2 cells transiently transfected with ERE, but glyphosate parent did not	Med	Parent-negative Formulations-positive	Gasnier et al. (2009)
In vivo: FSTRA		In this validated assay, the non-treatment-responsive decrease (only significant at mid-treatment) in VTG was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic		Med	Negative	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
		index, gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology				
In vivo: Rainbow trout VTG assay	Glyphosate and glyphosate plus surfactants; measured concentration of glyphosate 0.11 mg/L for 7 days	VTG induction in fish is a standard measure for estrogenicity in environmental regulatory toxicology that also considers the relevance to humans (e.g. USEPA FIFRA SAP 2009a,b, 2012). Glyphosate did not increase plasma VTG in levels in juvenile rainbow trout, glyphosates plus surfactants were only marginally greater than the controls, no trend, no significance		Med	Negative	Xie et al. (2005)
Overall conclusion: No convincing evidence of a potential interaction with the estrogen pathway. The one in vitro study that is positive has not been reproduced by another laboratory.						
Androgen pathway						
EDSP Tier 1 data 2014/2015: In vitro: negative; both for androgen-receptor binding assay and the aromatase assay In vivo mammalian assays: Hershberger and male pubertal assays	Glyphosate	Standardized and validated assays	Androgen-receptor binding assay is not a validated OECD TG but other validated androgen receptor assays not available in 2014/2015 Aromatase assay: highest soluble test concentration of glyphosate was 10^{-3} mol/L The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger and male pubertal assays) were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial	High	Negative, but sperm count and delay in preputial separation effects seen at very high doses, > 1 000 mg/kg bw per day	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			separation at 1 234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1 000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study)			
In vitro: hAR transactivation assay in CHO cells	Glyphosate (> 95–100%) formulation not specified in the paper Concentrations given for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.			Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hAR transient transfection into human HepG2 cells, aromatase evaluation within the HepG2 cells, and MDA-MB453-kb2 cells	Glyphosate and formulations Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line. Method for aromatase activity evaluation is also part of OECD TG 456 for steroidogenesis.	MDA-MB453-kb2 cell line has a high content of glucocorticoid receptors in addition to androgen receptors The characterization of the cell line and discussion of such confounding factors is not considered in the paper. While glyphosate and formulations reduced AR transcription in this cell line, there appears to have been no control with androgen-specific responses to exclude glucocorticoid-specific responses	Low	Positive	Gasnier et al. (2009)
Steroidogenesis In vitro: Transformed and human aromatase–transfected cDNA in human embryonic kidney 293 cells and placental-	Glyphosate and formulations 0.01% (with 210 µmol/L glyphosate) to 2% glyphosate/glyphosate formulation	Relevant cell models, but limited characterization provided in the paper	Inhibition of aromatase noted in two different species by both parent compound and formulations The aromatase assay may be subject to variability, e.g. due to degradation of the enzyme, and therefore performance criteria are specified in guideline OPPTS 890.1200 to	Low–Med	Positive	Benachour et al. (2007)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
derived JEG3 cells Ex vivo: normal human placenta and equine testis			demonstrate that the assay is functioning correctly. This is addressed in the EDSP data, but is not evident in the Séralini lab. papers (Benachour et al., 2007; Gasnier et al., 2009), although OECD GD 150 is cited. An adequate response with the proficiency chemicals econazole, fenarimol, nitrofen (inhibitors) and atrazine (non-inhibitor) should be demonstrated and the inhibitor 4-hydroxyandrostenedione (formestane) used as a positive control chemical in each experiment. While the correct positive control was used, proficiency testing is not reported Compliance with the performance criteria should be checked before evaluating results from this assay. A positive result in GD OPPTS 890.1200 requires demonstration of inhibition of aromatase activity that fits a 4-parameter nonlinear regression model such that the concentration response curve crosses 50% inhibition. The concentration response curve allows the determination of potency, i.e. IC_{50}. In some cases, variability may be due to limited solubility of a chemical			
Steroidogenesis In vitro: Placenta-derived JEG3 cells	Glyphosate and formulation ingredients Top dose: 100 ppm		The coformulants were each tested independently and were reported to inhibit aromatase activity at concentrations 20–67% below the no-observed-effect concentration, at which levels glyphosate alone did not significantly inhibit aromatase. (See also comment above regarding proficiency testing of the assay)	Low–Med	Positive	Defarge et al. (2016)
In vitro: BLTK1 murine Leydig cells	300 µmol/L	characterized Leydig cell model				(2012)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Steroidogenesis In vitro: StAR in a mouse MA-10 Leydig tumour cell	Glyphosate formulation (containing 180 g/L glyphosate)	Relevant and well-characterized cell model	Statistically significant reduction ($P < 0.01$) of $(Bu)_2$cAMP with the glyphosate formulation was observed after 2 hours of treatment. Statistical significance ($P < 0.01$) was also [illegible] for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones	Med–High	Positive	Walsh et al. (2000)
Overall conclusion: There is no convincing evidence of a potential interaction between glyphosate and the androgen-receptor pathway. Decreases in sperm count in the subchronic rat study (1 678 mg/kg bw per day; USEPA 2015) and a delay in preputial separation at 1234 mg/kg bw per day in the two-generation reproduction study in rats (the EDSP Tier 2 study) were observed at a dose that was above the limit dose (1000 mg/kg bw per day) and therefore of low physiological relevance. However, there is plausible evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. Further investigation is needed.						
Thyroid						
EDSP Tier 1 data 2014/2015: In vitro: No assays conducted. In vivo test battery: There were no treatment-related effects on T4 and TSH, thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. No thyroid-related effects were observed in the female pubertal assay. There were no developmental effects or alterations in thyroid histopathology in the amphibian metamorphosis assay. No thyroid-related effects were noted in any of the Part 158 studies.	Glyphosate	Relevant and validated test methods	No convincing evidence of potential interaction of glyphosate	High	Negative	USEPA 2015

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Overall conclusion: There is no convincing evidence of a potential interaction with the thyroid pathway for glyphosate						
Other endocrine mechanisms						
Retinoid system In vivo *Xenopus laevis* embryo model and chicken embryos	360 pg and 5 000 pg of glyphosate (Sigma)	Whole vertebrate models, two species	Experimental design and hypothesis based on medical observations of craniofacial defects with malformations observed in humans residing in areas chronically exposed to glyphosate formulations. Suspected to be resulting from a dysfunctional retinoic-acid or Sonic hedgehog pathway. Further investigation is needed	Med–High	Positive: increase in endogenous retinoic-acid activity	Paganelli et al. (2010)
Cortisol In vivo fish study *Rhamdia quelen* fingerlings	Glyphosate formulation 360 g/L	Stress response of *Rhamdia quelen* fingerlings acute exposure at 45, 90, 135 and 180 days	Stress responses important but difficult variable to control for, as stress is induced from handling, etc. This study included appropriate controls for stress confounders	Med	Negative for impaired cortisol release, but impaired growth and survival	Koakoski et al. (2014)
Hypolipidaemia and peroxisome proliferation In vivo rat	Glyphosate formulation 300 mg/kg single daily dose for 2 weeks, 5 animals/dose per group		No increase in number or size or peroxisomes	Med	Negative	Vainio et al. (1983)
AhR induction In vitro: Mouse hepatoma Hepa1c1c7 cells AhR Luciferase reporter gene transcriptional assay	Glyphosate (95–100% purity) Assay performed at concentrations of $\leq 10^{-5}$ mol/L	Relevant and recognized assay		Med	Negative	Takeuchi et al. (2008)
In vitro mPPARα, mAhR, hPXR	Glyphosate		Review, insufficient detail given. Concentration tested not given for negative test chemicals	Low	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
Overall conclusion: Suggestion of adverse effect upon retinoic-acid pathways. Further investigation required.						

AhR: aryl hydrocarbon receptor; AR: androgen receptor; CAS: Chemical Abstracts Service; CHO: Chinese hamster ovary; EDSP: Endocrine Disruptor Screening Program; ER: estrogen receptor; ERTA: estrogen receptor transcriptional activation; FSTRA: fish short-term reproduction assay; GD: guideline; hAR: human androgen receptor; HepG2: hepatocellular carcinoma; IC_{50}: median inhibitory concentration; no.: number; OECD: Organisation for Economic Co-operation and Development; PPAR: peroxisome proliferator-activated receptor; PXR: pregnane X receptor; rhCG: recombinant human chorionic gonadotrophin; StAR: steroidogenic acute regulatory protein; T4: thyroxine; TG: test guideline; TSH: thyroid-stimulating hormone; VTG: vitellogenin

[a] High: line of evidence could be sufficient on its own to be almost sure of entry (approaching 100% likelihood); Med: contributes importantly towards increasing likelihood; Low: minor contribution towards increasing likelihood.