UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: **June 29, 2015**

SUBJECT: **EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals**

PC Code: See table, Attachment A
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: See table, Attachment A
MRID No.: NA

DP Barcode: NA
Registration No.: NA
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CAS No.: NA
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EPA has completed its Weight of Evidence (WoE) assessment evaluating results of the Endocrine Screening Program (EDSP) Tier 1 screening assays for the List 1 chemicals. The WoE documents for the 52 chemicals are listed in Attachment A along with the chemical and report identifiers.

Attachment A. EDSP List 1 Chemicals

Chemical Name	PC Code	TXR Number
2,4-D	030001	0057151
Abamectin	122804	0057152
Acephate	103301	0057153
Acetone	044101	0057154
Atrazine	080803	0057155
Benfluralin	084301	0057156
Bifenthrin	128825	0057157
Captan	081301	0057158
Carbaryl	056801	0057159
Carbofuran	090601	0057160
Chlorothalonil	081901	0057161
Chlorpyrifos	059101	0057162
Cyfluthrin	128831	0057163
Cypermethrin	109702	0057164
DCPA	078701	0057165
Diazinon	057801	0057166
Dichlobenil	027401	0057167
Dimethoate	035001	0057168
EPTC	041401	0057169
Esfenvalerate	109303	0057170
Ethoprop	041101	0057171
Fenbutatin-Oxide	104601	0057172
Flutolanil	128975	0057173
Folpet	081601	0057174
Glyphosate	417300	0057175
Imidacloprid	129099	0057176
Iprodione	109801	0057177
Isophorone	847401	0057178
Linuron	035506	0057179
Malathion	057701	0057180
Metalaxyl	113501	0057181
Methomyl	090301	0057182
Metolachlor	108801	0057183
Metribuzin	101101	0057184
MGK-264	057001	0057185
Myclobutanil	128857	0057186
Norflurazon	105801	0057150
o-Phenylphenol	064103	0057146
Oxamyl	103801	0057142
PCNB	056502	0057138
Permethrin	109701	0057149
Phosmet	059201	0057145
Piperonyl Butoxide	067501	0057141
Pronamide	101701	0057137
Propargite	097601	0057148
Propiconazole	122101	0057144
Pyriproxyfen	129032	0057140
Simazine	080807	0057136
Tebuconazole	128997	0057143
Tetrachlorvinphos (TCVP)	083701	0057147
Triadimefon	109901	0057139
Trifluralin	036101	0057135

EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS

CHEMICAL: GLYPHOSATE

OFFICE OF PESTICIDE PROGRAMS

OFFICE OF SCIENCE COORDINATION AND POLICY

U.S. ENVIRONMENTAL PROTECTION AGENCY

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Abbreviations

Abbreviation	Terminology
A	Androgen (hormonal pathway)
ADME	Absorption, Distribution, Metabolism, Excretion
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMA	Amphibian Metamorphosis Assay
ARTA	Androgen Receptor Transcriptional Activation
AST	Aspartate Aminotransferase
ANOVA	Analysis of Variance
AOP	Adverse Outcome Pathway
AR	Androgen Receptor
B_{max}	Binding at maximum
BROD	Benzyloxyresorufin-O-dealkylase
BUN	Blood Urea Nitrogen
CAR	Constitutive Androstane Receptor
CFR	Code of Federal Regulations
CG	Cowper's Gland
ChE	Cholinesterase
ChEI	Cholinesterase inhibition
CMC	Carboxymethyl cellulose
CTA	Comparative Thyroid Assay
CV	Coefficient of Variation
CYP	Cytochrome 450
DER	Data Evaluation Record
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DP	Dorsolateral Prostrate
E	Estrogen hormonal pathway
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDRT	Endocrine Disruptor Review Team
EDSP	Endocrine Disruptor Screening Program
EE	Ethinyl Estradiol
ELISA	Enzyme Linked Immunosorbent Assay
EOGRTS	Extended One-Generation Reproductive Toxicity Study (Rat)
ER	Estrogen Receptor
EROD	Ethoxyresorufin-O-dealkylase (or deethylase)
ERTA	Estrogen Receptor Transcriptional Activation
EtOH	Ethanol
F	Female
F1	First filial generation

Abbreviation	Terminology
F2	Second filial generation
Fcd	Fecundity
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Field Observation Battery
FQPA	Food Quality Protection Act
Frt	Fertility
FSH	Follicle Stimulating Hormone
FSTRA	Fish Short-Term Reproduction Assay
FT	Flutamide
GD	Gestation Day
GGT	Gamma-glutamyl Transpeptidase
GnRH	Gonadotropin-releasing hormone
GP	Glans Penis
GSI	Gonado-Somatic Index
H	High
HLL	Hind Limb Length
HPG	Hypothalamic-Pituitary-Gonadal Axis
HPLC/MS/MS	High Pressure Liquid Chromatography/Mass Spectroscopy
HPT	Hypothalamic-Pituitary-Thyroidal Axis
I	Inadequate
IC50	Inhibitory Concentration at 50% of response
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative
K_d	Equilibrium Dissociation Constant
K_{ow}	Octanol/Water Partition Coefficient
L	Low dose
LABC	Levator Ani-Bulbocavernosus
LAGDA	Larval Amphibian Growth and Development Assay
LC50	Lethal Concentration in 50% of test organisms
LD	Lactation Day
LH	Luteinizing hormone
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Limit of Quantitation
M	Male
MDL	Minimum Detection Level
MEOGRT	Medaka Extended One Generation Reproduction Test
MH	Medium high
ML	Medium low
MoA	Mode of Action
MOE	Margin of Exposure
MRID	Master Record Identifier
MROD	Methoxyresorufin-O-dealkylase
MTC	Maximum Tolerated Concentration

Abbreviation	Terminology
MTD	Maximum Tolerated Dose
N	Negative
NE	Not examined/evaluated
NF stage	Nieuwkoop and Faber's Staging Atlas
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NS	Not Statistically Significant
NR	Not Reported
OCSPP	Office of Chemical Safety Pollution and Prevention
OECD	Organization for Economic Co-Operation and Development
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSCP	Office of Science Coordination and Policy
OSRI	Other Scientifically Relevant Information
P	Positive
P	Parental generation
PC	Positive Control
PC_{10}	Positive Control at 10% of response
PC_{50}	Positive Control at 50% of response
PND	Post-Natal Day
POD	Point of Departure
PPS	Preputial Separation
PROD	Pentaoxyresorufin-O-dealkylase (or depentylase)
PXR	Pregnane X receptor
QC	Quality Control
RBA	Relative Binding Affinity
RBC	Red Blood Cells
RfD	Reference Dose
RPC_{max}	Relative to Positive Control at maximum
SAP	Scientific Advisory Panel
SC	Solvent Control
s.c	Subcutaneous
SDH	Sorbitol dehydrogenase
SDWA	Safe Drinking Water Act
SEP	Standard Evaluation Procedure
SD	Standard Deviation or Sprague-Dawley
SVL	Snout-to-Vent Length
SV	Seminal Vesicles
T	Thyroid (hormonal pathway)
T1WoERC	EDSP Tier 1 Weight of Evidence Review Committee
T3	Triiodothyronine
T4	Thyroxine (tetraiodothyronine)

Abbreviation	Terminology
TP	Testosterone Propionate
TR	Thyroid Receptor
TSH	Thyroid Stimulating Hormone
UDPGT	Uridine Diphosphate Glucuronyltransferase (also known as UGT)
VC	Vehicle Control
VO	Vaginal Opening
VP	Ventral Prostate
VTG	Vitellogenin
WoE	Weight-of-Evidence

Executive Summary

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality, were considered in this weight of evidence (WoE) assessment.

In determining whether glyphosate interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude of responses, and the pattern of responses observed across studies, taxa, and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

On September 17, 2014, the EDSP Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of-evidence (WoE) analysis of the potential interaction of glyphosate with the E, A or T hormone pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 *in vitro* assays followed by *in vivo* mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

For the estrogen pathway, while glyphosate showed estrogen receptor (ER) antagonism *in vitro* with estrogen-dependent human breast cancer cells (Thongprakaisang *et al*., 2013), there was no evidence of potential interaction of glyphosate with the estrogen pathway in the EDSP Tier 1 *in vitro* assays [*i.e,* ER binding, ER transactivation assay (ERTA), aromatase and steroidogenesis assays]. Additionally, glyphosate was negative in the Tier 1 *in vivo* mammalian assays (*i.e.,* uterotrophic or female pubertal assays). In the fish short-term reproduction assay (FSTRA), the non-treatment-responsive decrease [only significant at mid-treatment] in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related endpoints such as gonado-somatic index (GSI), gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology. There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies (decreases in offspring body weight observed in one avian reproduction study). Therefore, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate.

For the androgen pathway, there was no evidence of interaction of glyphosate with the androgen pathway in the Tier 1 *in vitro* [*i.e*, androgen receptor (AR) binding and steriodogenesis assays

were negative] or Tier 1 *in vivo* FSTRA and mammalian assays (*i.e*., Hershberger and male pubertal assays were negative in the absence of overt toxicity). In addition, glyphosate was negative in an AR transactivation assay (Kojima *et al.*, 2004). 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/day) and a delay in preputial separation (PPS)at 1234 mg/kg/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 (1000 mg/kg/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).

For the thyroid pathway, there was no convincing evidence of potential interaction of glyphosate. There were no treatment-related effects on thyroid hormones (T4 and TSH), thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. There were no thyroid-related effects observed in the female pubertal assay. In the amphibian metamorphosis assay (AMA), there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.

Based on weight of evidence considerations, mammalian or wildlife EDSP Tier 2 testing is not recommended for glyphosate since there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways.

I. Introduction

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality, were considered in this weight of evidence (WoE) assessment.

In determining whether a pesticide chemical interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude of responses, and the pattern of responses observed across studies, taxa and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

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Glyphosate has a vapor pressure of 9.8×10^{-8} mm Hg at 25°C, with a water solubility of 10.5 g/L at 20°C. The octanol/water partition coefficient is log $K_{ow} = -3.2$ to -3.4 with fish bioconcentration factors ranging from 0.2-0.63. Based on its vapor pressure and low Henry's Law constant, glyphosate has low potential to volatilize from soils or from water. Glyphosate adsorbs strongly to soil and is stable to chemical and photochemical decomposition and hydrolysis; however, it is readily degraded by soil microbes.

The available information considered in determining the potential interaction of glyphosate with the E, A and/or T pathways include submitted EDSP Tier 1 assays and/or other scientifically relevant information (OSRI) such as general toxicity studies and other published articles. These data are summarized in Sections III.A through III.C. An analysis of the data submitted to the Agency, using the WoE approach outlined by the Agency (USEPA, 2011), is presented in Section IV. The EDSP Tier 2 studies recommendations are presented in Section V.

II. Sources of Scientific Data and Technical Information

A. EDSP Tier 1 Screening Assays

The Tier 1 assays and/or other scientifically relevant information (OSRI) submitted to satisfy the agency's test order are shown below in Table 1. Executive Summaries are presented in Appendix 1.

B. Other Scientifically Relevant Information (OSRI)

In response to the Agency's Test Orders, data believed to be relevant to one or more of the Tier 1 assays were submitted as OSRI by the Test Order recipients and/or the public. This included studies published in the open literature and/or data submitted to support pesticide registration (*e.g.*, Part 158 guideline studies), and a review of the available scientific literature conducted by Health Canada's Pest Management Regulatory Agency (USEPA, 2014). The Agency's review of the initial OSRI is provided in the Report of the Endocrine Disruptor Review Team on OPP (USEPA, 2010). Since then, the Agency has also conducted a more recent search of available scientific literature for any additional relevant information. Summaries of the available OSRI are presented in Appendix 2. Additionally, literature/studies considered but not utilized for the WoE analysis are listed in Appendix 3.

III. Weight of Evidence (WoE) Evaluation

The principles, criteria and approach used in the WoE determination on the potential of a substance to interact with endocrine-related processes (*i.e.,* E, A, or T hormonal pathways) were as described in the WoE guidance document (USEPA, 2011) and presented at the 2013 FIFRA Scientific Advisory Panel (SAP) (USEPA, 2013). The weight of evidence process identifies how the individual lines of evidence are assembled and integrated along two concepts (*i.e.*, complementarity and redundancy) within the conceptual framework of an adverse outcome pathway). Broadly, there are four main steps outlined in the guidance which provide the foundation for WoE evaluations. The first step is to evaluate the individual studies for their scientific quality and relevance in evaluating potential endocrine interaction(s). The second step is to integrate the data along different levels of biological organization while examining the extent of complementarity (*i.e*., the concordance of endpoints within an assay that measures multiple endpoints) and redundancy (*i.e*., the concordance of endpoints/responses across assays) in the observed responses across these different levels of biological organization. The third step is to characterize the main lines of evidence as well any conclusions. Finally, the last step is to evaluate whether additional testing is needed based on the evidence and conclusions described above.

As mentioned, the first step is to assemble and evaluate the available scientific data. Data for the EDSP Tier 1 WoE evaluation falls into one of two categories: 1) EDSP Tier 1 data, or 2) other

scientifically relevant information (OSRI). The EDSP Tier 1 data include a battery of 11 assays consisting of *in vitro* and mammalian and wildlife *in vivo* assays. The Tier 1 assays were designed specifically to evaluate a number of key biological events including potential effects on receptor binding (estrogen and androgen agonist and antagonist), steroidogenesis, and other effects on the HPG and HPT axes. OSRI may include published literature studies as well as studies conducted under USEPA (often referred to as Part 158 data) or OECD guidelines submitted in support of pesticide registrations. Each study is evaluated for scientific quality and relevance for informing interactions with the E, A, or T pathway. Additionally, the consistency of the responses in the individual study is evaluated. For the Tier 1 *in vivo* assays, often multiple endpoints are measured in each assay.

Evaluation of the potential confounding effects of overt toxicity in the study, as well as the relative degree of diagnostic utility of a specific endpoint for discerning whether or not the chemical has interacted with the endocrine system, are considered. The collective response of the individual endpoints, as well as the conditions under which they were expressed, are considered when evaluating an overall indication of potential interaction as measured by the study.

The second step in this WoE process is to formulate hypotheses and integrate the available data along different levels of biological organization. Two keys elements in the integration of data, as well as characterizing the extent to which the available data support a hypothesis that a chemical has the potential to interact E, A or T pathways, are the concepts of complementarity and redundancy. These two concepts provide a basis for considering the plausibility, coherence, strength, and consistency of the body of evidence. The current EDSP Tier 1 screening assays are meant to evaluate whether or not a chemical can interact with E, A and T consisting of different levels of biological organization from a molecular initiating event, such as receptor binding, through potential adverse effects in apical endpoints such as sexual development and fecundity at the whole organism level. The extent of expression of responses at higher levels of biological organization can indirectly provide information on potential compensatory capabilities of an individual organism.

After the data have been assembled and integrated, the third step is to characterize the main lines of evidence along with the conclusions; this characterization involves three components. The first component is whether the data provide relevant, robust and consistent evidence in terms of complementarity and redundancy as well as biological plausibility. Second, is at what level of biological organization were the responses observed and whether organisms exhibit compensatory responses at higher levels of biological organization? Finally, under what conditions dide the responses occur including consideration of whether the responses were observed in the presence of overt or systemic toxicity? The presence of overt and/or systemic toxicity introduces uncertainty in the ability to distinguish effects specifically related to an endocrine-related effect from a non-endocrine toxic response.

This uncertainty in distinguishing whether the responses were endocrine-related was discussed at the FIFRA SAP meeting that evaluated scientific issues associated with the WoE evaluation of the EDSP Tier 1 screening process. In October 2013, the SAP stated that , "*In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis"* (USEPA, 2013).

For these WoE analyses, overt toxicity was generally defined in accordance with EPA's current approach as used by OPP in reviewing 40 CFR Part 158 studies for both human and ecological risk assessments. Specifically, in these analyses, the effects that EPA considered to be potential evidence of overt toxicity included, but were not limited to: mortality; clinical signs such as tremors, ataxia and abnormal swimming (fish and aquatic-phase amphibians); and body weight decreases of ≥10% in mammals. Additionally, other effects including morphological (*e.g.*, organ weights/histopathology), biochemical (*e.g.*, blood chemistry), and other clinical signs (*e.g.*, lethargy) were also considered when evaluating overt toxicity, especially if the effects were extreme. In some instances, one parameter (*i.e.*, death or >10% decrease in mammalian body weight) was sufficient to consider a dose/concentration to be overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. For example, in the FSTRA, generally, body weight decreases were considered along with other responses when assessing potential overt toxicity. Additionally, effects which were considered to be signs of systemic toxicity were also captured and these effects were generally considered as less severe forms of toxicity (*e.g.*, organ weights or blood chemistry). The circumstances for which a dose/concentration was considered overtly toxic for a particular study are described in Section IV.A.

In summary, EPA considers multiple lines of evidence in including the observed responses in the Tier 1 assays and OSRI in the context of a chemical's physical/chemical properties and its known modes of action in its overall characterization of a chemical's potential to interact with the E, A or T pathway. Adequately addressing the three components described above is fundamental to the WoE process and in determining whether additional data are needed. In addition to characterizing the WoE, reviewers also considered: 1) uncertainties and their potential impact to conclusions; 2) discussion of key studies; 3) description of inconsistent or conflicting data; 4) overall strength of evidence supporting a conclusion; and, 5) what, if any, additional data are needed and why. Assessing the need for additional data is based on a case-by-case analysis which took all available toxicity data into account.

The WoE approach involved consideration of data (i.e., lines of evidence) from the EDSP Tier 1 assays and OSRI which are depicted in **Tables 2 - 4**. *These tables contain data that are considered scientifically and biologically relevant with regard to a treatment-related effect which supports a conclusion of whether a substance has the potential to interact with the E, A,*

or T pathway. Effects that occurred in the presence of overt toxicity are discussed in the text for each respective pathway (E, A or T) but are not reported in the table for E, A or T.

A. EDSP Tier 1 Screening Assays

The Tier 1 assays submitted in response to the agency's test order for glyphosate are shown below in **Table 1**.

Table 1 Tier 1 Screening Assays for Glyphosate

Tier 1 Assays	Test Guideline	Test Order Status
ER Binding Assay (Rat uterine cytosol)	OSCPP 890.1250	Requirement Satisfied (MRID 48671305)
ERα Transcriptional Activation Assay (Human cell line HeLa 9903)	OSCPP 890.1300; OECD 455	Requirement Satisfied (MRID 48671307)
AR Binding Assay (Rat prostate cytosol)	OSCPP 890.1150	Requirement Satisfied (MRID 48671301)
Steroidogenesis Assay (Human cell line H295R)	OSCPP 890.1550; OECD 456	Requirement Satisfied by OSRI (Hecker *et al*., 2011)
Aromatase Assay (human recombinant microsomes)	OSCPP 890.1200	Requirement Satisfied (MRID 48671303)
Uterotrophic Assay (Rat)	OSCPP 890.1600; OECD 440	Requirement Satisfied (MRID 48677003)
Hershberger Assay (Rat)	OSCPP 890.1400; OECD 441	Requirement Satisfied (MRID 48617001)
Pubertal Female Assay (Rat)	OSCPP 890.1450	Requirement Satisfied (MRID 48671315)
Pubertal Male Assay (Rat)	OSCPP 890.1500	Requirement Satisfied (MRID 48671313)
Fish Short-term Reproduction Assay	OSCPP 890.1350; OECD 229	Requirement Satisfied (MRID 48671311)
Amphibian Metamorphosis Assay (Frog)	OSCPP 890.1100; OECD 231	Requirement Satisfied (MRID 48671309)

B. Effects on Hypothalamic-Pituitary-Gonadal (HPG) Axis

1. Effects on Estrogen Pathway

The potential for glyphosate to interact with the estrogen pathway is summarized in Table 2. The various targets of the estrogen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for estrogenic, anti-estrogenic or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

Glyphosate was negative (or not interactive) in the *in vitro* estrogen receptor (ER) binding, aromatase, and steroidogenesis assays. In the Tier 1 ERα transcriptional activation (ERTA), the data for the very weak agonist control chemical (17α-methyltestosterone) did not respond appropriately in the assay, and therefore, this study was not considered reliable for evaluating potentially very weak agonists. However, it is unlikely that glyphosate is an estrogen receptor agonist because (1) there was no activity in an ERTA assay in which the strong and weak agonist reference chemicals (17β-estradiol and 17α-estradiol, respectively) showed the expected activity, and (2) negative responses were observed in the Tier 1 ER binding and uterotrophic assays, which also assess estrogen receptor-related activity. In a literature study by Thongprakaisang *et al.*, 2013, glyphosate induced ERE activation in T47D-KBluc cells. Co-incubation of glyphosate with ICI 182780 (ER antagonist) resulted in a decreased or inhibited response in these cells.

The uterotrophic assay was negative up to the limit dose (1000 mg/kg/day). No estrogen-related effects were observed in the female pubertal rat assay up to the limit dose (1000 mg/kg/day). No estrogen-related effects were seen in the mammalian Part 158 studies.

In the FSTRA, there was a significant decrease in plasma vitellogenin (VTG) concentrations in the medium high treatment group (6.2 mg a.i/L) as compared to the negative control. This reduction from control was 55%, with non-dose-dependent decreases in VTG concentrations of approximately 30-33% at all other treatment concentrations. Other female-related endpoints, including gonado-somatic index (GSI), gonadal staging, fecundity, and fertilization success were not significantly affected. Additionally, there were no notable histopathological diagnoses that were attributed to glyphosate exposure.

In available Part 158 avian reproduction studies (two with the mallard duck and one with the bobwhite quail), there were no treatment-related effects on all reproductive parameters (tested up to 1000 mg/kg-diet). In another mallard duck reproduction study, test up to 2250 mg/kg-diet, reductions in 14-d offspring body weight were observed at 501 mg/kg-diet as well as reductions in male body weight; there were no other effects observed.

In a OSRI fish study (Le Mer *et al.*, 2013) with wild larval three-spined sticklebacks (*Gasterosteus aculeatus*), there was no VTG induction observed in fish treated with glyphosate up to 10 µg/L. Additionally, there were no effects on histology including proportions of intersex individuals. In a Part 158 fish life-cycle toxicity study with fathead minnow, there was no treatment-related effect on any of the reproductive or growth parameters up to 25.7 mg/L.

Table 2. Estrogenic/Anti-Estrogenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormone	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Vitellogenin (VTG)	Systemic Toxicity Obsserved[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assays															
ER Binding (MRID 48671305)	N														
ERTA (MRID 48671307)		N													
Aromatase (MRID 48671303)			N												
Steroidogenesis (MRID 48617005)			N												
Uterotrophic (MRID 48617003)					N									N	N
Female Pubertal Rat (MRID 48671315)					N	N	N	N	N	N				X (M, H)[4]	N
FSTRA (MRID 48671311)				NE		N	N				N	N	N		N
OSRI															
Receptor Transactivation Assay (Kojima *et al.*, 2004)		N													
Receptor Transactivation Assay (Thongprakaisang *et al.*, 2013)		P[5]													
90-Day Subchronic Toxicity (Rat; MRID 40559401)						NE	N	NE						N	N

Table 2. Estrogenic/Anti-Estrogenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormone	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Vitellogenin (VTG)	Systemic Toxicity Obsserved[2]	Overt Toxicity Observed[3]
90-Day Subchronic Toxicity (Rat; NTP, 1992)						NE	N	NE						X (H)	N
90-Day Subchronic Toxicity (Mouse; MRID 00036803)						N	N	NE						X (H)	N
90-Day Subchronic Toxicity (Mouse; NTP, 1992)						NE	N	NE	N					X (H)	X (H)
Developmental Toxicity (Rat; MRID 00046362)					NR							N		X (H)	X
Developmental Toxicity (Rabbit; MRID 00046363)					NR							N		X (M, H)	X
Three-Generation Reproduction (Rat; MRIDs 00081674, 00105995)					NE	N	N	N	NE			N		N	N
Two-Generation Reproduction (Rat; MRID 41621501)					NE	N	N	NE				N		X (H)	X (H)
Two-Generation Reproduction (Rat; MRIDs 48865101-48865105)					N	N	N	N		N		N		X (H)	N

Table 2. Estrogenic/Anti-Estrogenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormone	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Vitellogenin (VTG)	Systemic Toxicity Obsserved[2]	Overt Toxicity Observed[3]
Chronic Toxicity (Dog; MRID 00153374)					NE	N	N	N						N	N
Carcinogenicity (Mouse; MRID, 00130406)					NR	N	N	NR						N	N
Chronic Toxicity/ Carcinogenicity (Rat; MRIDs 00093879, 00150564)					NE	N	N	N						X (L, M)	N
Chronic Toxicity/ Carcinogenicity (Rat; MRID 41643801)					NE	NE	N	NE						X (H)	N
Avian Reproduction (Duck; MRID 00036328; 00111953)												N		N	N
Avian Reproduction (Duck; MRID 48876602)												P[6]		N	N[7]
Avian Reproduction (Quail; MRID 00108207)												N		N	N
Early Life-Stage Toxicity (Sticklebacks; Le Mer *et al.*, 2013)													N		N
Fish Life Cycle Toxicity (MRID 00108171)												N			N

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment
   Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that this parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated. I= Inconclusive; LW= Liver weight; I= inconclusive
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Rales was observed in medium- and high-dose animals (4/15 and 13-15, respectively) at approximately 4 hours post-dose.
5. Glyphosate caused hormone-dependent T47D cells to proliferate, approximately 15-30% of control, in the absence of E2. Glyphosate also induced ERE activation by 5- to 13-fold in T47D-KBluc cells compared to control. Both responses were inhibited in the presence of ICI 182780.
6. Reductions in 14-d offspring body weight at 501 mg/kg-diet. No effects on egg production or embryo viability were observed.
7. Reduction in male body weight gain at 501 mg/kg-diet; no effects on female body weight gain or other clinical signs of toxicity.

P Positive findings
N Negative (*in vitro*)/No effect (*in vivo*)
NE Not examined
NR Not reported

2. Effects on Androgen Pathway

The potential for glyphosate to interact with the androgen pathway is summarized in **Table 3.** The various targets of the androgen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for androgenic, anti-androgenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

Glyphosate was negative (or a non-binder) in the *in vitro* androgen receptor (AR) binding (rat prostate cytosol) and steroidogenesis assay.

Glyphosate was also classified as negative in the *in vivo* Hershberger assay as there were no treatment-related effects in accessory sex tissue (AST) weights up to the limit dose (1000 mg/kg/day). Glyphosate had no effect on androgenic endpoints in the FSTRA, including GSI, gonadal histology and staging, as well as no effects on VTG.

In the male pubertal assay, decreases were observed in the weights of the ventral prostate, seminal vesicle, LABC, pituitary glands as well as a 2.1 day delay in PPS at the high dose (1000 mg/kg/day) but were concurrent with significant decreases in body weight (10%). Additionally, there was no treatment-related changes in serum testosterone concentrations or any histopathological changes in any tissues in at any dose.

In the Part 158 two-generation rat reproduction study, a delay in attainment of PPS, and increased body weight at attainment, occurred in F_1 male pups at 1234 mg/kg/day. In the 90-day subchronic rat study, relative testes weight was increased 21% at a dose of 3393 mg/kg/day which occurred in the presence of overt toxicity (↓18% body weight); no changes were observed in absolute weights. Additionally, sperm counts were decreased at 1678 and 3393 mg/kg/day; however, no changes in testes histopathology were observed. In the 90-day mouse study, relative testes weight were increased 10 and 18% at a dose of 4776 and 10,780 mg/kg/day, respectively, which was concurrent with decreases in body weight greater than 10%. In the carcinogenicity study in mice, relative testes weights were increased (17.5%; NS) at a dose (4500 mg/kg/day) which is approximately four-fold higher than the limit dose.

In the fish study by Le Mer *et al.*, 2013, there was no effect on the induction of spiggin (a protein secreted by males to construct nests) observed in any of the treatment groups. Additionally, while effects on male body weight gain and offspring body weight were observed in one Part 158 mallard duck reproduction study, there were no other androgen-related effects observed in the Part 158 avian reproduction studies or fish life-cycle study.

Table 3. Androgenic/Anti-Androgenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin (VTG)	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assays															
AR Binding (MRID 48671301)	N														
Steroidogenesis (MRID 48617005)			N												
Hershberger (MRID 48617001)				NE						N				N	N
Male Pubertal Rat (MRID 48671313)				N	N	N	N	N	N	N		N		X[4]	BW: ↓10% (H)
FSTRA (MRID 48671311)				NE	N	N				N	N		N	NE	N
OSRI															
Receptor Transactivation Assay (Kojima *et al.*, 2004)		N													
90-Day Subchronic Toxicity (Rat; MRID 40559401)					N	N	N	N	NE					N	N
90-Day Subchronic Toxicity (Rat; NTP, 1992)					N	N	N	N	NE		P[5]			X (H)	X (H)

Table 3. Androgenic/Anti-Androgenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin (VTG)	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
90-Day Subchronic Toxicity (Mouse; MRID 00036803)					N	N	NE	N	NE					X (H)	N
90-Day Subchronic Toxicity (Mouse; NTP, 1992)					N	N	N	N	NE					X (M, MH, H)	X (MH, H)
Three-Generation Reproduction (Rat; MRIDs 00081674, 00105995)					N	N			N		N			N	N
Two-Generation Reproduction (Rat; MRID 41621501)					N	N	N	N	N		N			X (H)	X (H)
Two-Generation Reproduction (Rat; MRIDs 48865101-48865105)					N	N	N	N	N	N	N	Age: ↑2.9 d Wt: ↑10% (H)[6]		N	N
Chronic Toxicity (Dog; MRID 00153374)					N	N	NE	NR	N					N	N
Carcinogenicity (Mouse; MRID 00130406)					N	N	NR	NR	NR					X (H)	N

Table 3. Androgenic/Anti-Androgenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin (VTG)	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Chronic Toxicity/ Carcinogenicity (Rat; MRIDs 00093879, 00150564)					N	N	NE	N	N					N	N
Chronic Toxicity/ Carcinogenicity (Rat; MRID 41643801)					N	N	N	N	NE					X (H)	N
Avian Reproduction (Duck; MRID 00036328)											N			N	N
Avian Reproduction (Duck; MRID 00111953)											N			N	N
Avian Reproduction (Duck: MRID 48876602)											P[7]			N	N[8]
Avian Reproduction (Quail; MRID 00108207)											N			N	N
Early Life-Stage Toxicity (Sticklebacks; Le Mer *et al.*, 2013)													N[9]		N

Table 3. Androgenic/Anti-Androgenic Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Glyphosate[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility(Frt) /Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin (VTG)	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Fish Life Cycle Toxicity (MRID 00108171)											N				N

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that this parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated. Abbreviations for androgen sensitive tissues: Seminal vesicles (SV), Ventral prostate (VP), Dorsal prostate (DP), Levator ani-bulbocavernosus (LABC), Epididymides (E), Cowper's gland (CG), glans penis (GP).; BW= Body weight; BWG: Body weight gain; LW= Liver weight
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Rales were observed in 9/15 and 14/15 males in the medium- and high-dose groups, respectively, approximately 4 hours post-dosing. This finding persisted during the daily examinations in 7/15 high-dose males throughout the study.
5. Sperm counts were decreased at 1678 mg/kg/day in the absence of overt toxicity; however epididymal sperm motility, total spermatid head/testis, and total spermatid heads/g caudal tissue in the treated animals were similar to control.
6. Delay in attainment of PPS, and increased body weight at attainment, occurred in F1 male pups at 1234 mg/kg/day.
7. Reductions in 14-d offspring body weight at 501 mg/kg-diet. No effects on egg production or embryo viability were observed.
8. Reduction in male body weight gain at 501 mg/kg-diet; no effects on female body weight gain or other clinical signs of toxicity
9. No induction of spiggin (a protein secreted by males to construct nests) in fish

P Positive findings
N Negative (*in vitro*)/No effect (*in vivo*)
NE Not examined
NR Not reported

C. Effects on Hypothalamic-Pituitary-Thyroidal (HPT) Axis

The current EDSP Tier 1 battery does not have a specific *in vitro* assay to detect chemicals with the potential to affect hypothalamic or pituitary regulation of thyroid hormone production, but it does include three *in vivo* assays that provide data to detect changes in the HPT axis, ***i.e.***, the pubertal female and male (rat) assays, and the AMA (frog).

The potential for glyphosate to interact with thyroid regulation is summarized in **Table 4.** The various targets of the thyroid pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for thyroid or HPT axis effects. This table also includes HPT-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

In the AMA, there were no effects on median NF developmental stage and, no asynchronous development was observed. There was a significant decrease ($p<0.05$) in Day 7 normalized hind limb length at the middle treatment concentration (4.3 mg a.i/L) but this effect was not observed at Day 21 along with no significant ($p>0.05$) effects at any other concentration for Days 7 and 21. There was no treatment-related effects on thyroid histopathology. There was a significant increase ($p<0.05$) in Day 21 snout-vent-length (SVL) in the middle, middle-high and high treatment concentrations (↑5.2, 2.5 and 6.8%, respectively). Additionally, there was a significant increase in Day 21 body weight at the highest treatment concentration (↑17%). In a study examining the chronic effects of glyphosate and its formulations to four North American frog species (Howe et al, 2004), treatment with glyphosate alone did not reveal any effects on growth (length), tail morphology, percent surviving to reach Gosner Stage 42, time to metamorphosis, gonadal development or thyroid gene expression relative to controls. It is noted that control mortality was relatively high in this study under chronic exposure (38%), and as such is not included in table below.

There were no thyroid-related effects observed in the female pubertal assay. In the male pubertal assay, while total thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations were decreased compared to control in all glyphosate treatment groups, they were not statistically significant and were not considered to be treatment-related. Additionally, there was a slight increase in the number of high-dose males with decreased colloid area that occurred in the presence of overt toxicity (↓10% body weight), and there were no treatment-related effects on follicular cell height at any dose compared to control. Furthermore, there were no effects on thyroid function observed in any of the mammalian Part 158 toxicity studies.

Table 4. Thyroid Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Thyroid Pathway for Glyphosate[1]									
Study Type/ Literature Citation	Thyroid Weight	Thyroid: Gross and Histopathology	Serum T_4 Levels	Serum TSH Levels	Pituitary Weight	Developmental stage (± or asynchronous, HLL)	Growth (BW, BL, SVL)	Systemic Toxicity [2]	Overt Toxicity[3]
EDSP Tier 1 Assays									
Female Pubertal Rat (MRID 48671315)	N	N	N	N	N			P (M, H)[4]	N
Male Pubertal Rat (MRID 48671313)	N	N	N	N	N			P (M, H)[5]	BW: ↓10% (H)
AMA (Frog; MRID 48671309)		N				N	Day 21: SVL: ↑5, 3,7% (M, MH, H)[6]; BW: ↑17% (H)[7]		N
OSRI									
90-Day Subchronic Toxicity (Rat; MRID 40559401)	NE	N			NE			N	N
90-Day Subchronic Toxicity (Rat; NTP, 1992)	NE	N			NE			X (H)	X (H)
90-Day Subchronic Toxicity (Mouse; MRID 00036803)	NE	N			NE			X (H)	N
90-Day Subchronic Toxicity (Mouse; NTP, 1992)	NE	N			NE			X (M, MH, H)	X (MH, H)
Three-Generation Reproduction (Rat; MRIDs 00081674, 00105995)	NE	N			N			N	N
Two-Generation Reproduction (Rat; MRID 41621501)	NE	NE			NE			X (H)	X (H)
Two-Generation Reproduction (Rat; MRIDs 48865101-48865105)	N	N			N			X (H)	N
Chronic Toxicity (Dog; MRID 00153374)	N	N			N			N	N

Table 4. Thyroid Pathway for Glyphosate

Lines of Evidence Indicating Potential Interaction with the Thyroid Pathway for Glyphosate[1]									
Study Type/ Literature Citation	Thyroid Weight	Thyroid: Gross and Histopathology	Serum T_4 Levels	Serum TSH Levels	Pituitary Weight	Developmental stage (± or asynchronous, HLL)	Growth (BW, BL, SVL)	Systemic Toxicity [2]	Overt Toxicity[3]
Carcinogenicity (Mouse; MRID, 00130406)	N	N			N			X (H)	N
Chronic Toxicity/ Carcinogenicity (Rat; MRIDs 00093879, 00150564)	N	N			N			X (L, M)	N
Chronic Toxicity/ Carcinogenicity (Rat; MRID 41643801)	NE	N			NE			X (H)	N

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that this parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated.
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Rales was observed in medium- and high-dose animals (4/15 and 13-15, respectively) at approximately 4 hours post-dose.
5. Rales were observed in 9/15 and 14/15 males in the medium- and high-dose groups, respectively, approximately 4 hours post-dosing. This finding persisted during the daily examinations in 7/15 high-dose males throughout the study.
6. Snout-vent length was increased in a non-dose-dependent manner in the top three dose groups on Day 21.
7. Body weight was increased 17% in the high-dose group on Day 21.

P Positive findings
N Negative findings
NE Not examined

IV. Committee's Assessment of Weight of Evidence

This section of the document describes the weight of evidence (WoE) determination on the potential of glyphosate to interact with endocrine related processes (*i.e.*, E, A, or T hormonal pathways) as well as recommendations regarding Tier 2 testing. The results of the Tier 1 assays are considered, along with other scientifically relevant information (*e.g.*, 40 CFR Part 158 test guidelines and published or publicly available peer-reviewed studies). WoE analysis in the context of the EDSP follows the Agency's guidance (USEPA 2011) and is conducted on a case-by-case basis by first assessing the different lines of evidence (*i.e.*, specific Tier 1 assays and OSRI), then performing an integrated analysis of those lines of evidence.

The WoE evaluation includes considerations of biological plausibility of the findings from the different lines of evidence by examining the consistency, coherence, and interrelationships among the measured endpoints within and across studies. The available findings from standard toxicology studies on the substance may contribute to the WoE evaluation in helping elucidate if effects seen in the Tier 1 assay are related to perturbations of the endocrine system *per se* or alternatively sequelae of systemic effects. Endocrine modes of action may elicit a number of phenotypic consequences other than those evaluated in the Tier 1 assays.

Endocrine-related findings in the presence of overt toxicity may result in uncertainty as to whether or not the responses are related through an endocrine pathway, therefore non-endocrine toxic responses (including but not limited to mortality or body weight changes) are also considered in this WoE evaluation. The FIFRA SAP that evaluated scientific issues associated with weight of evidence evaluation of the results of the Tier 1 assays stated that "*In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis*" (USEPA, 2013).

A. Systemic/Overt Toxicity in the *in vivo* Tier 1 Assays and OSRI

Effects that were considered to be systemic or overt toxicity for the *in vivo* Tier 1 and OSRI studies are described below. Generally, one parameter (i.e., death or >10% decrease in mammalian body weight) is sufficient for a dose/concentration to be considered overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. Effects which were considered to be signs of systemic toxicity were generally less severe forms of toxicity (e.g., changes in organ weights or blood chemistry).

1. Tier 1 *in vivo* Assays

In the Hershberger assay and the uterotrophic assays, all animals survived until scheduled termination, and no treatment-related clinical findings were noted in any of the animals during the study. In the female pubertal rat assay, one animal in the control group was sacrificed *in extremis* on PND 27 due to impairment of the right forelimb (due to possible mechanical injury); all other animals survived until scheduled sacrifice. In the male pubertal rat assay, one high-dose male was found dead prior to dosing on PND 24; no significant clinical or macroscopic findings were observed in this animal, with all other rats surviving until scheduled sacrifice. Treatment-related clinical signs in the pubertal studies were limited to rales in 4/15 females and 9/15 males at the medium dose (approximately 4 hours post-dosing), and in 13/15 females and 14/15 males at the high dose. This finding did not persist to the daily examinations at the medium dose, but were present in the daily examinations in 7/15 males at the high dose throughout the study. No other treatment-related clinical signs were noted during the 4-hour post-dosing or daily examinations at any dose level. Final body weights were decreased by 7 and 10% in the medium- and high-dose males. Moderate decreases in liver weights ($p<0.05$; ↓10 and 15%) also were also noted in the medium- and high-dose males, but considered secondary to decreased body weights.

In the FSTRA, survival was 100% in the negative control and all treatment groups, except the medium-dose treatment group (one male mortality). Glyphosate did not result in any significant changes in weight or length for either sex at any treatment level. There were no observed effects on secondary sex characteristics and clinical signs (*i.e.*, behavioral and other sub-lethal effects) in male or female fish at any treatment group. Additionally, glyphosate exposure also did not affect the survival of tadpoles at any concentration in the AMA, and no clinical signs of toxicity were observed.

2. OSRI

In a subchronic oral toxicity study in rats conducted by the National Toxicology Program (NTP; 1992), all animals survived to necropsy. Diarrhea was noted in the high-dose animals through Day 50 of the study. Body weight gain was reduced in the high-dose males (3393 mg/kg/day), and the final body weight was decreased 18%, but statistical significance was not reported. Mean necropsy body weights were decreased in the 1678 and 3393 mg/kg/day males (↓12 and 21%, respectively). Body weight gain in high-dose females was decreased, but no effects on final or necropsy body weights were observed. Non-dose-dependent increases in relative liver weights also were observed in males in all dose groups and in females in the two highest dose groups. These findings, combined with clinical chemistry changes, are consistent with a hepatobiliary effect that can be attributed to glyphosate administration.

In a subchronic oral toxicity study in mice, there were no treatment-related toxicological signs or mortalities during the study and no effects of treatment on food consumption. Body weight gains in the high-dose male and female groups were decreased compared to control.

In a subchronic oral toxicity study in mice conducted by NTP (1992), there were two non-treatment-related mortalities in the study. In addition, no clinical signs of toxicity were reported. Final body weights were decreased in males and females in the two highest dose groups (↓>10% for males and ≤10% for females) with concomitant decreases in body weight gain; statistical significance was not reported. Mean necropsy body weights were decreased for the medium-high (4776 and 5846 mg/kg/day, respectively) and high-dose (10,780 and 11,977 mg/kg/day, respectively) males and females. Non-dose-dependent increases in relative liver weight also were noted in males of all dose groups, except at the low dose. A decrease in absolute liver weight also was noted in high-dose females.

In a developmental toxicity study in rats, there were six deaths in the high-dose group, but none of the deaths were determined to be related to treatment. At the high-dose, the majority of the dams (all but three) were observed at least once with diarrhea, soft stools, breathing rattles, inactivity, and red matter in the region of nose, mouth, forelimbs, or dorsal head. No treatment-related clinical signs were observed in the low- and mid-dose groups; additionally, there were no treatment-related effects on maternal body weight in these groups. At the high dose, maternal body weight gain was decreased during the test period (GDs 0-20) due to body weight losses during GDs 6-20 (≤10%). In a developmental toxicity study in rabbits, five rabbits aborted and were sacrificed. Additionally, rabbits at the medium and the high dose died during the study; of these, five deaths were determined to not be related to treatment, but the cause of death for the remaining eight rabbits could not be determined at necropsy. Soft stool or diarrhea was noted in all groups, with a slight increase at the medium dose and at least one incidence in each rabbit of the high-dose group. In addition, an increase in nasal discharge was noted in the high-dose animals. There were no toxicologically significant differences in maternal mean body weight among control and treated groups.

There were no treatment-related mortalities in a two-generation reproduction study with rats. Treatment-related clinical signs consisted of soft stools in high-dose males and females (both F_0 and F_1). Body weights of high-dose male and female F_0 rats were decreased (<10%) during the pre-mating growth period. Similarly, F_1 high-dose male and female adults weighed less at weaning compared to F_0 animals with continued decreases of 10-12%. The body weights of high-dose females remained decreased compared to control during gestation and lactation of the F_1, F_2a, and F_2b litters, although the high-dose group body weights were comparable to control by the end of lactation. Food consumption was generally similar to control in high-dose males and females during most measurement periods; consumption was decreased in the medium- and high-dose F_1 females on pre-mating Days 0-14 (↓9 and 11%, respectively). Terminal body weights in the F_0 and F_1a generations were decreased by 8-13%. In a subsequent two-generation reproduction study with rats, there were no treatment-related effects on clinical signs or mortality. No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, or food consumption. Absolute and relative (to body) liver weights were

increased by 8-13% in both the P and F1 females at in the high-dose group with no histopathological findings.

In a chronic oral toxicity/carcinogenicity study in rats, survival was unaffected by treatment. Decreased body weight gains were observed in treated males beginning at Week 26 and continuing to Week 102, and decreased body weight gains were present in the low- and medium-dose female rats from Weeks 84 to 92. No dose-response relationship was apparent in the decreased body weight gains in female rats, and the changes in body weight did not affect survival. In a subsequent chronic oral toxicity/carcinogenicity study in rats, there were also no treatment-related effects on clinical signs or mortality. The mean body weight of high-dose females was decreased by 13% at Week 81, with decreased a body weight gain of 23% compared to control. There were no decreases in body weight or body weight gain in male rats. Liver weight (relative to body weight) was increased (↑13%) in high-dose males at interim sacrifice, and absolute liver weights were increased (↑13%) in high-dose males at terminal sacrifice. In a carcinogenicity study in mice, there were no treatment-related toxic signs noted during the study, and mortality was low during the first eighteen months of the study. Body weight was consistently decreased for males and, to a lesser extent, females at the high-dose (4500 mg/kg/day) during the study at several sampling intervals, with no treatment-related changes in body weight at the low- and mid-dose groups.

No treatment-related mortalities, clinical signs of toxicity, or body weight/body weight gain changes were observed in a subchronic oral toxicity study in rats, a three-generation reproduction study in rats, a chronic toxicity study in dogs, or the avian reproduction studies in mallard ducks and bobwhite quail. In another mallard duck reproduction study, reductions in male body weight gain were observed at 501 mg/kg-diet and 2250 mg/kg-diet.

B. Estrogen Pathway

While glyphosate showed ER antagonism *in vitro* with estrogen-dependent human breast cancer cells (Thongprakaisang et al., 2013), there was no evidence of potential interaction of glyphosate with the estrogen pathway in the Tier 1 *in vitro* assays (*i.e,* ER binding, ERTA, aromatase and steroidogenesis assays). Additionally, glyphosate was negative in the Tier 1 *in vivo* mammalian assays (*i.e.,* uterotrophic or female pubertal assays). In the fish short-term reproduction assay (FSTRA), the non-treatment-responsive decrease [only significant at mid-treatment] in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related endpoints such as gonado-somatic index (GSI), gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology. There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies (decreases in offspring body weight observed in one avian reproduction study). Therefore, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate.

C. Androgen Pathway

There was no evidence of interaction of glyphosate with the androgen pathway in the Tier 1 *in vitro* (*i.e*, AR binding and steriodogenesis assays were negative) or Tier 1 *in vivo* FSTRA and mammalian assays (*i.e*., Hershberger and male pubertal assays were negative in the absence of overt toxicity). In addition, glyphosate was negative in an AR transactivation assay (Kojima *et al.*, 2004). 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/day) and a delay PPS in the post 1998 2-generation reproduction study (1234 mg/kg/day) in the rat. Both effects were observed at a dose that was above the limit dose (1000 mg/kg/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).

D. Thyroid Pathway

There was no convincing evidence of potential interaction of glyphosate with the thyroid pathway. There were no treatment-related effects on thyroid hormones (T4 and TSH), thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. There were no thyroid-related effects observed in the female pubertal assay. In the AMA, there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.

E. Conclusions

The conclusion of the WoE evaluation is that glyphosate demonstrates no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways in mammals or wildlife.

V. EDSP Tier 2 Testing Recommendations

Based on weight of evidence considerations, mammalian or wildlife EDSP Tier 2 testing is not recommended for glyphosate since there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways.

VI. References

Beavers, J. and Fink, R. (1978) Final Report: One-generation Reproduction study-Bobwhite Quail: Glyphosate Technical: Project No. 139-141. (Unpublished study received Nov 13, 1978 under 524-308; prepared by Wildlife International, Ltd., submitted by Monsanto Co., Washington, DC; CDL: 235924-B). (MRID 00108207)

Beavers, J. and Fink, R. (1978) Final Report: One-generation Reproduction Study-Mallard Duck: Glyphosate Technical: Project No. 139-143. (Unpublished study received Nov 13, 1978 under 524-308; prepared by Wildlife International Ltd., submitted by Monsanto Co., Washington, DC; CDL:235924-A). (MRID 00111953)

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2007) Glyphosate technical: dietary two generation reproduction study in the rat. Unpublished study conducted by Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, November 6, 2007. (MRID 48865101)

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2012) Glyphosate technical: dietary two generation reproduction study in the rat; cortical vacuolation historical data. Unpublished study conducted by Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, June 18, 2012. (MRID 48865105)

EG & G, Bionomics. (1975) Chronic Toxicity of Glyphosate to the Fathead Minnow (*Pimephales promelas*, Rafinesque). (Unpublished study received Dec 27, 1978 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:097759-B). (MRID 00108171)

Frey, L.T. 2012. Glyphosate-acid: A Reproduction Study with the Mallard *(Anas platyrhynchos).* Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Study No. 123-187. Study sponsored by Syngenta Crop Protection, LLC, Greensboro. NC. Study initiated May 26, 1998 and completed January 11, 1999. (MRID 48876602).

Hecker, M., Hollert H., Cooper, R., *et al*. (2011) The OECD validation program of the H295R steroidogenesis assay: phase 3. Final inter-laboratory validation study. MRID 48617005. Published: Environ. Sci. Pollut. Res. (2011) 18:503-515.

Howe, **C.** M, M. Berrill, B. D. Pauli, C. C. Helbing, K. Werry and N. Veldhoen. 2004. *Toxicity of Glyphosate-based Pesticides to Four North American Frog Species.* Society of Environmental Toxicology and Chemistry 23(8): 1928 - 1938.

Kojima, H., Iida, M., Katsura, E., Kanetoshi, A., Hori, Y., and Kobayashi, K. (2003) Effects of a diphenyl ether-type herbicide, chlornitrofen, and its amino derivative on androgen and estrogen receptor activities. *Environ. Health Perspect.* 111:497–502.

Kojima, H., Katsura, E., Takeuchi, S., Niiyami, K., and Kobayashi, K. (2004) Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ. Health Perspect.* 112 (5): 524-531.

Knezevich, A. and Hogan, G. (1983) A Chronic Feeding Study of Glyphosate (Roundup Technical) in Mice: Project No. 77-2061: BDN-77420. Final rept. (Unpublished study received Aug 17, 1983 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, DC; CDL:251007-A; 251008; 251009; 251010; 251011; 251012;251013;251014). (MRID 00130406)

Lankas, G.R. and Hogan, G.K. (1981) A Lifetime Feeding Study of Glyphosate (Roundup Technical) in Rats: Project No. 772062. (Unpublished study received Jan 20, 1982 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, D.C.; CDL:246617-A; 246618; 246619; 246620; 246621). (MRID 00093879)

Le Mer, C., Roy, R.L., Pellerin, J., Couillard, C.M., and Maltais, D. (2013) Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (*Gasterosteus aculeatus*). *Ecotoxicol. Environ. Saf.* 89: 174-181.

McConnel, R. (1985) A Chronic Feeding Study of Glyphosate (Roundup Technical in Mice): Pathology Report on Additional Kidney Sections: Addendum to Final Report Dated July 21, 1983: Project No. 77-2061A. Unpublished study prepared by Bio/dynamics Inc. 59 p. (MRID 00150564)

National Toxicology Program. (1992) *Technical Report on Toxicity Studies of Glyphosate (CAS No. 1071-83-6) Administered in Dosed Feed to F344/N Rats and B6C3Fl Mice*, Toxicity Report Series Number 16, NIH Publication 92-3135, July 1992. U.S. Department of Health and Human Services, Research Triangle Park, NC.

Reyna, M. (1985) Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs: Project No. ML-83-137: Study No. 830116. Unpublished study prepared by Monsanto Company Environmental Health. 317 p. (MRID 00153374)

Reyna M. (1990) Two Generation Reproduction Feeding Study with Glyphosate in Sprague Dawley Rats: Lab Project No: MSL-10387, Study No. ML 88-106. Unpublished study prepared by Monsanto Agricultural Co. (MRID 41621501)

Rodwell, D.E., Tasker, E.J., Blair, A.M. *et al.* (1980) Teratology Study in Rabbits: IRDC No. 401056, Study No. IR 79-018. (Unpublished study; prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL: 242516-B). (MRID 00046363)

Rodwell, D.E., Tasker, E.J. Blair, A.M. *et al.* (l980) Teratology Study in Rats: IRDC No. 401-054, Study No. IR 79-016. (Unpublished study including IRDC no. 999-021; received May 23, 1980 under 524-308; prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL:242516-A. (MRID 00046362)

Schneider, S.Z., Kendall, T.Z., and Krueger, H.O. (2012) Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances. Unpublished study conducted by Wildlife International, Ltd., Easton, Maryland 21601. Laboratory report number 707A-103. Study sponsored by Joint Glyphosate Task Force c/o Data Group Management, Inc., Raleigh, North Carolina 27615. Study completed April 11, 2012. (MRID 48671309)

Schneider, S.Z., Martin, K.H., Kendall, T.Z., and Krueger, H.O. (2012) Glyphosate: Fish Short-Term Reproduction Assay with the Fathead Minnow (*Pimephales promelas*). Unpublished study conducted by Wildlife International, Ltd., Easton, MD 21601. Laboratory report number 707A-102A. Study sponsored by Joint Glyphosate Task Force c/o Data Group Management, Inc., Raleigh, NC 27615. Study completed April 11, 2012. (MRID 48671311)

Schroeder, R.E. and Hogan, G.K. (1981) A Three-Generation Reproduction Study with Glyphosate in Rats: Project No. 77-2, Study No. BDN 77-417 (Unpublished study received Sep 22,1981 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, D.C.; CDL: 245909-A). (MRID 00081674)

Stout, L. and Johnson, C. (1987) 90-day Study of Glyphosate Administered in Feed to Sprague Dawley Rats: Proj. ID ML-86-351/EHL 86128. Unpublished study prepared by Monsanto Agricultural Co. (MRID 40559401)

Stout, L. and Ruecker, F. (1990) Chronic Study of Glyphosate administered in Feed to Albino Rats: Lab Project Number: MSL-I0495: R.D. 1014, Study No. ML 87-148. Unpublished study prepared by Monsanto Agricultural Co. (MRID 41643801)

Street, R. (1982) Letter sent to R. Taylor dated Jul 6, 1982: Roundup Herbicide: Addendum to pathology report for a three-generation reproduction study in rats with glyphosate. (Unpublished study received Jul 7, 1982 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:247793-A). (MRID 00105995)

Street, R.W., Conkin, R.A., Edwards, G.A., *et al.* (1980) A Three-Month Feeding Study of Glyphosate in Mice: Special Report# MSL-1154. (Unpublished study received Jul 2, 1980 under 524308; submitted by Monsanto Co., Washington, D.C.; CDL:242799. (MRID 00036803)

Stump, D. G. (2012) A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats. Unpublished study conducted by WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No.: WIL-843003, January 6, 2012. (MRID 48617001)

Stump, D.G. (2012) A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats. Unpublished study conducted by WIL Research Laboratories, LLC, Ashland, OH, Laboratory Project ID: WIL-843007, April 10, 2012. (MRID 48671315)

Stump, D.G. (2012) A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats. Unpublished study conducted by WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-843005, April 10, 2012. (MRID 48671313)

Stump, D. G. (2012) A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats. Unpublished study conducted by WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No.: WIL-843002, January 6, 2012. (MRID 48617003)

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., and Satayavivad, J. (2013) Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem. Toxicol*. 59:129-136.

USEPA (2010) Glyphosate: Report of the Endocrine Disruptor Review Team; Test Order #: EDSP- 417300-229; 230; 240; 241; 244; 246; 247; and 248, December 16, 2010. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency, Washington, DC.

USEPA (2011) Endocrine Disruptor Screening Program – Weight of Evidence: Evaluating Results of EDSP Tier 1 Screening to Identify the Need for Tier 2 Testing. Office of Chemical Safety and Pollution Prevention Science Coordination and Policy. U.S. Environmental Protection Agency, Washington, DC.

USEPA (2013) Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held July 30 - August 1, 2013 on Weight-of-Evidence: Evaluating Results of EDSP Tier 1 Screening. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency, Washington, DC.

USEPA (2014) Glyphosate: Literature Search Review. March 7, 2014. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency, Washington, DC.

Wilga, P.C. (2012) Glyphosate: Human Recombinant Aromatase Assay. Unpublished study conducted by CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100334AROM, March 9, 2012. (MRID 48671303)

Willoughby, J.A. (2012) Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate. Unpublished study conducted by CeeTox, Kalamazoo, MI. Laboratory Report No.: 6500V-100334ERTA, March 8, 2012. (MRID 48671307)

Willoughby, J.A. (2012) Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay. Unpublished study conducted by CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100334ARB, March 8, 2012. (MRID 48671301)

Willoughby, J.A. (2012) Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol). Unpublished study conducted by CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100364ERB, March 8, 2012. (MRID 48671305)

Willoughby, J.A. (2012) Supplemental Information - Laboratory Proficiency Data for ERTA assays and Saturation Binding Data for AR and ER Binding Assays for Assorted Chemicals. Unpublished study conducted by CeeTox, Inc., Kalamazoo, MI. July 19, 2011. MRID 48843501)

APPENDIX 1. EDSP Tier 1 Screening Assays

Amphibian Metamorphosis Assay (AMA); (OCSPP 890.1100)

The 21-day assay (MRID 48671309) of glyphosate (85.14% purity) on amphibian metamorphosis of African clawed frog (*Xenopus laevis*) was conducted under flow-through conditions. Amphibian larvae at Nieuwkoop-Faber (NF) stage 51 (80 per control and treatment group) were exposed to nominal concentrations of 0 (negative control), 0.16, 0.80, 4.0, 20, and 100 mg a.i./L. Mean-measured concentrations were <0.100 (<LOQ; control), 0.13, 0.79, 4.3, 20, and 90 mg a.i./L. The test system was maintained at 21.4 to 22.3°C and a pH of 7.0 to 8.3.

The survival of tadpoles exposed to glyphosate was not significantly affected ($p>0.05$) as it was 100% in the control group and 99% in each of the treatment concentrations. Tail curvature was the only observed clinical sign (i.e., behavioral and other sublethal effects) at test termination and occurred in 46 to 57 (out of 80 exposed) tadpoles in the control and treatment groups (64, 63, 65, 53, 53, and 78% of tadpoles in the negative control, 0.13, 0.79, 4.3, 20, and 90 mg a.i./L treatment groups, respectively), and did not appear to be treatment-related.

Glyphosate caused no significant acceleration or delay of median NF developmental stage throughout the test. Further, no asynchronous development was observed. No tadpoles in the control and treatment groups developed beyond NF stage 57. Glyphosate exposure did not cause significant effects on Day 7 or 21 normalized (for snout-vent length) hind-limb length (HLL) at any concentration tested except a significant decrease ($p<0.05$) at the mid (4.3 mg a.i/L) concentration (↓15.6%) at Day 7. This effect was not observed in any treatment group at Day 21. Snout-vent length (SVL) was not significantly affected at any treatment concentration at Day 7 but was significantly increased ($p<0.05$) in the 4.3, 20, and 90 mg a.i/L treatment concentrations at Day 21 (↑5.2, 2.5, and 6.7%, respectively) compared to the control. Additionally there was a significant increase in Day 21 body weight at 90 mg a.i./L (↖ 17%).

There were no treatment-related effects on thyroid gland histopathology at any treatment level, with comparable incidence and severity of thyroid gland atrophy and hypertrophy, and follicular cell hypertrophy and hyperplasia in the control and treatment concentrations. While there appeared to be an increased incidence of mild thyroid gland hypertrophy at the highest treatment concentration, the same incidence was observed at the lowest treatment concentration and the effect was not concentration responsive. Similar findings were observed for follicular cell height increase: an apparent increase in mild severity at the top concentration with a similar incidence at the lowest treatment concentration and no concentration-responsive pattern. Finally, the pathologist report indicated that there were no treatment-related changes in the thyroid glands of tadpoles exposed to glyphosate when compared to those in the negative control.

Androgen Receptor (AR) Binding Assay; (OCSPP 890.1150)

In an androgen receptor (AR) binding assay (MRID 48617301), ventral prostate cytosol from Sprague Dawley rats was used as the source of AR to conduct saturation and competitive binding experiments. The saturation binding experiment was conducted to demonstrate that the AR in the rat prostate cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radio-labeled reference androgen (R1881). The competitive binding assay measured the binding of a single concentration of [^{3}H]-R1881 (1 nM) in the presence of increasing concentrations (logarithmic increase from 10^{-10} to 10^{-3} M) of glyphosate (95.93% glyphosate acid, 85.14% calculated glyphosate content, Batch # GLP-1103-21149-T). Low-salt TEGD buffer was used as the vehicle for glyphosate. A total of 3 runs were performed, and each run included dexamethasone as a weak positive control, and R1881 as the ligand reference standard.

Saturation binding data were not originally provided in the study report; however, summarized saturation binding data (MRID 48843501) from the performing laboratory were submitted following a request by the Agency. The dissociation constant (K_d) for [^{3}H]-R1881 was 0.613±0.041 nM and the estimated B_{max} was 0.817±0.049 fmol/100 µg protein for the single batch of prostate cytosol that was prepared. The mean and individual K_d values were below the range reported in the EPA validation program (0.685 to 1.57 nM). Confidence in these numbers is high according to the goodness of fit (R^2 = 0.957-0.984) and the small variation among runs.

In the competitive binding experiment, the estimated mean log IC_{50}s for R1881 and the weak positive control (dexamethasone) were −9.0 and −4.6 M, respectively, and the mean relative binding affinity (RBA) for the weak positive control, dexamethasone, was 0.004%. Confidence in the numbers for the reference standards is high as the values as variation between runs was small. All performance criteria were met.

At glyphosate concentrations of 10^{-10} to 10^{-3} M, specific binding of [^{3}H]-R1881 was 92.4-101.3% with the exception of one concentration (10^{-9} M) in Run 1, which had an average binding of 66.5%. Review of the data indicated that this value was a result of a single replicate with a specific binding of 7.5%. Excluding this value yielded a mean specific binding of 96.0%, which concurs with the other runs. As the specific [^{3}H]-R1881 binding was >75% at all concentrations of glyphosate in all runs, an IC_{50} and RBA could not be calculated for glyphosate.

Based on the results from the three runs, glyphosate is classified as a Non-Binder in the Androgen Receptor Binding Assay.

Aromatase Assay; (OCSPP 890.1200)

In an *in vitro* aromatase (CYP 19) assay (MRID 48671303), glyphosate (95.93% glyphosate acid; 85.14% calculated glyphosate content in sample; Lot # GLP-1103-21149-T) was incubated with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H(N)]-androst-4-ene-3,17-dione; [^{3}H]ASDN) at log concentrations of 10^{-10} to 10^{-3} M for 15 minutes to assess the potential of glyphosate to inhibit aromatase activity. The solvent vehicle was 0.1 M phosphate buffer for glyphosate, ethanol for ASDN, and dimethyl sulfoxide (DMSO) for 4-OH ASDN, with a final assay volume of ≤1% DMSO.

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting (LSC). Four independent runs were conducted; however, the first run was not used because of incorrect standard preparation. The remaining three runs were conducted and each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} M) with a known inhibitor (4-hydroxyandrostenedione; 4-OH ASDN), and the test chemical series (10^{-10} to 10^{-3} M) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.676 ± 0.072 nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90 to 110% of the average full activity. Activity in the background controls ranged 0.23 to 0.38% and averaged 0.30% of the full activity control. The response of the full activity controls and background controls was acceptable for each run.

For the positive control substance (4-OH ASDN), aromatase activity results were within the recommended ranges for the performance criteria. The estimated log IC_{50} for 4-OH ASDN averaged −7.29 M and the Hill slope was −0.96.

For glyphosate, aromatase activity averaged 0.673 ± 0.066 nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration of 10^{-10} M and 0.741 ± 0.100 nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration of 10^{-3} M. The average aromatase activity was ≥99.67% of the control at all tested glyphosate concentrations for all runs.

Based on the data from the average response curve, glyphosate is classified as a Non-inhibitor of aromatase activity in this assay.

Estrogen Receptor (ER) Binding; (OCSPP 890.1250)

In an estrogen receptor (ER) binding assay (MRID 48671305) for glyphosate (95.93% glyphosate acid, 85.14% calculated glyphosate content, Batch # GLP-1103-21149-T), uterine cytosol from Sprague Dawley rats was used as the source of ER to conduct saturation and competitive binding experiments. A saturation binding experiment was conducted to demonstrate that the ER in the rat uterine cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radio-labeled reference estrogen prior to conducting ER competitive binding experiments. The competitive binding experiment measured the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) in the presence of increasing concentrations (10^{-10} to 10^{-3} M) of glyphosate. TEGD buffer was used as the solvent vehicle for glyphosate. A total of 3 runs were performed, and each run included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control, and 17-β-estradiol as the natural ligand reference material.

Saturation binding data were not originally provided in the study report; however, summarized saturation binding data (MRID 48843501) from the performing laboratory were submitted following a request by the Agency. The protein concentrations used in the saturation binding runs varied between each run, and were approximately 3- to 6-fold greater than recommended (160 to 320 µg versus 50±10 µg). The K_d for [^{3}H]-17β-estradiol was 0.331 ± 0.061 nM and the estimated Bmax was 74.55 ± 3.03 fmol/100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected Guideline range of 0.03 to 1.5 nM.

In the competitive binding experiment, the estimated mean log IC_{50}s for 17β-estradiol and 19-norethindrone were −9.0 and −5.5 M, respectively. The mean relative binding affinity (RBA) was 0.032% for 19-norethindrone, compared to the natural ligand.

Glyphosate was tested over a concentration range (10^{-10} to 10^{-3} M) that fully defined the top of the curve. The percent binding at the top plateau (101.2-116.9%) was within 25 percentage points of the lowest concentration of the estradiol standard (98.6-101.8%). Across all runs, the lowest average percent radiolabeled estradiol binding in the presence of glyphosate was >81% (*i.e.* showed less than 25% displacement) at concentrations up to 10^{-3} M indicating that gylphosate was not competing with the natural ligand for binding to the ER.

Based on the results from the three runs, glyphosate is classified as Not Interactive in the Estrogen Receptor Binding Assay.

ERα Transcriptional Activation (ERTA) Assay; (OCSPP 890.1300)

In an estrogen receptor transcriptional activation assay (MRID 48617307), hERα-HeLa-9903 cells cultured *in vitro* were exposed to glyphosate (85.14% a.i. Batch # GLP-1103-21149-T) at logarithmically increasing concentrations from 10^{-10} to 10^{-3} M in cell culture media for 24 hours in three independent runs. The experiments were performed using 96-well plates and each glyphosate concentration was tested in 6 wells/plate in each run. The solvent vehicle was culture media for glyphosate and DMSO (0.1%) for the reference chemicals. Cells were exposed to the test agent for 24±2 hr to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor was measured using a proprietary luciferase assay.

Glyphosate was tested up to the limit dose, with no precipitation or cytotoxicity observed at any tested concentration. At concentrations up to 10^{-3} M, the relative transcriptional activation of glyphosate was ≤2.4%. In the main assays, the responsiveness of the cells to the very weak positive control 17α-methyltestosterone was lower than the expected values, indicating a decreased sensitivity of the assay to very weak agonists. Although the conditions of this assay were not optimal to detect very weak activity, glyphosate responses were similar to those of the negative control corticosterone and not comparable to the responses of 17α-methyltestosterone, which was able to reach a maximum of 40.8-42.6% PC. Glyphosate was only able to reach a maximum of 0.8-2.4% PC when tested up to the highest concentration possible based on cytotoxicity. Because the $RPC_{Max} < PC_{10}$ in both assay runs, glyphosate was considered negative for estrogen receptor transcriptional activation in this test system.

Fish Short-Term Reproduction Assay (FSTRA); (OCSPP 890.1350)

The 21-day short-term reproduction assay (MRID 48671311) of glyphosate with Fathead minnow (*Pimephales promelas*) was conducted under flow-through conditions. Adult fish (2 males and 4 females in each group, 4 groups/treatment, 5.5 months of age) were exposed to glyphosate (85.14% purity) at nominal concentrations of 0 (negative control), 0.048, 0.24, 1.2, 6.0, and 30 mg a.i./L. Mean-measured concentrations were <0.03 (<LOQ; negative control), 0.046, 0.23, 1.2, 6.2, and 33 mg a.i./L. The high test concentration was based on 1/3 of a 96-hr LC50 value. The test system was maintained at 24.3 to 29.1°C and a pH of 8.0 to 8.3.

Survival was 100% in the negative control, 0.046, 0.23, 6.2 and 33 mg a.i./L treatment groups and 95.8% in the 1.2 mg a.i./L treatment group. Glyphosate did not result in any significant increases or decreases in weight or length for either sex at any treatment level. There were no observed effects on secondary sex characteristics or clinical signs (i.e., behavioral and other sublethal effects) in males or females in any treatment group.

Spawning and mean fecundity in the negative control were at least every 4 days in each replicate and 23.5 eggs/female/day/replicate (range: 23.2-23.9 eggs/female/day), respectively; fertilization success in the negative control was 97.3%. Fecundity and fertilization success were not significantly different from the negative control for any treatment group.

Plasma vitellogenin (VTG) was significantly decreased 55% ($p<0.05$) in female fish at the mid high-treatment level (6.2 mg a.i./L) compared to the negative control; male VTG was unaffected by treatment. There were no effects in gonado-somatic index (GSI) or nuptial tubercle scores for male or females (none noted in females) in any treatment group relative to the negative control. Although there were gonadal histopathology observations reported for both males and females (e.g. minimal to mild granulomatous inflammation, oocyte atresia, increased mature oocytes), there were no treatment-related patterns in gonadal histopathology for males or females.

Hershberger Assay; (OCSPP 890.1400)

In a Hershberger Assay (MRID 48617001) screening for androgenic activity, glyphosate (85.1% a.i., Batch/lot# GLP-1103-21149-T) in 0.5% methylcellulose (w/v) was administered daily via oral gavage (5 mL/kg) to groups of six 54- or 55-day old, castrated male Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 (limit dose) mg/kg/day. The androgenic positive control group consisted of 6 castrated rats exposed to 0.2 mg/kg/day of testosterone propionate (TP) by subcutaneous (s.c.) injection.

To screen for potential anti-androgenic activity, glyphosate in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six 54- or 55-day old, castrated male Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 mg/kg/day in conjunction with a daily dose of reference androgen TP at 0.2 mg/kg/day by s.c. injection. The anti-androgenic positive control group consisted of 6 castrated rats exposed to 0.2 mg/kg/day TP by s.c. injection and 3 mg/kg/day flutamide (FT) via oral gavage. TP alone was used as the anti-androgenic negative control.

For both components of the assay, body weights were determined daily. The animals were dosed for 10 consecutive days and terminated approximately 24 hours after the final dose. At necropsy, the five androgen-dependent tissues were collected and weighed.

All animals survived until scheduled termination. No animals exhibited any dose-related clinical signs of toxicology and there were no treatment-related gross pathological findings.

In the androgen agonist assay, there were no treatment-related effects on body weights, overall body weight gains, or the weights of accessory sex organs for any glyphosate dose group. Animals in the positive TP control group had increased ($p<0.01$) accessory sex organ weights as

follows: 437% in seminal vesicles; 728% in ventral prostate; 200% in levator ani-bulbocavernosus (LABC); 361% in Cowper's gland; and 45% in glans penis. The performance criteria indicated that this assay was performing as expected.

In the anti-androgen assay, there were no treatment-related effects on body weights, overall body weight gains, or the weights of accessory sex organs for any glyphosate dose group. Animals dosed with TP + FT (positive control) had decreased ($p<0.01$) accessory sex organ weights as follows: 76% in seminal vesicles; 80% in ventral prostate; 63% in LABC; 70% in Cowper's gland; and 29% in glans penis. The performance criteria indicated that this assay was performing as expected.

Statistically significant changes were not seen in two or more of the five androgen sensitive tissue weights. Glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay.

Female Pubertal Assay; (OCSPP 890.1450)

In a Female Pubertal Assay (MRID 48671315), 15 Crl:CD(SD) Sprague-Dawley rats/dose group were treated daily via oral gavage with glyphosate technical (95.93% a.i., Lot #: GLP-1103-21149-T) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg/day (limit dose) from post-natal day (PND) 22 to 42. Animals were examined for vaginal opening (VO) daily beginning on PND 22, and age and weight at day of attainment were recorded. Following sacrifice on PND 42, blood was collected for clinical chemistry analyses, including total thyroxine (T_4) and thyroid stimulating hormone (TSH) levels, which were analyzed using an electrochemiluminescent immunoassay (T_4) and a magnetic [^{125}I]rTSH gamma counter immunoassay (TSH). Liver, adrenal glands, thyroid, pituitary, and urogenital organ weights were recorded, and microscopic examinations were performed on the thyroid, kidneys, ovary and uterus.

One animal in the control group was sacrificed *in extremis* on PND 27 due to impairment of the right forelimb (due to possible mechanical injury). All other animals survived until scheduled sacrifice. Treatment-related clinical signs were limited to rales in 4/15 and 13/15 females in the 300 and 1000 mg/kg/day groups, respectively, at approximately 4 hours post-dosing. This finding did not persist to the daily examinations. No other treatment-related clinical signs were noted during the 4-hour post-dosing or daily examinations at any dose level. There were no treatment-related differences in age of attainment of VO, body weight at VO, final body weights, or body weight gains in the treated groups relative to controls. One female each in the control and 300 mg/kg/day groups failed to attain VO. There were no statistically significant differences in mean age at first vaginal estrus, mean cycle length, or percent cycling. The cycle status at necropsy was similar among all groups. Serum T_4 and TSH were not affected by treatment, and

no adverse treatment-related effects on any clinical chemistry parameter were observed at any dose. There were no treatment-related microscopic findings in the thyroid, ovaries, uterus, or kidneys at any dose.

The dose levels tested were adequate since the high dose was the Limit Dose (1000 mg/kg/day).

Male Pubertal Assay; (OCSPP 890.1500)

In a Male Pubertal Assay (MRID 48671313), 15 Crl:CD(SD) Sprague-Dawley rats/dose group were treated daily via oral gavage (5 mL/kg) with glyphosate technical (95.93% a.i., Lot #: GLP-1103-21149-T) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg/day (limit dose) from post-natal day (PND) 23 to 53. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and weight at day of attainment were recorded. Following sacrifice on PND 53, blood was taken for total thyroxine (T_4), testosterone, thyroid stimulating hormone (TSH), and clinical chemistry analysis. The hormones were analyzed by radioimmunoassay (RIA) or chemiluminescence. Weights were recorded for the liver, adrenal glands, thyroid, pituitary, and urogenital organs, and microscopic examinations were performed on the thyroid, kidneys, right testis and epididymides.

One male in the 1000 mg/kg/day group was found dead prior to dosing on PND 24; no significant clinical or macroscopic findings were observed in this animal. All other rats survived until scheduled sacrifice. Treatment-related clinical findings were limited to rales in 9/15 and 14/15 males in the 300 and 1000 mg/kg/day groups, respectively, approximately 4 hours post-dosing. This finding persisted in the daily examinations in 7/15 males at 1000 mg/kg/day throughout the study.

Treatment-related decreases in overall (PND 23-53) body weight gains were observed at 300 mg/kg/day (↓8%, not significant; NS) and 1000 mg/kg/day (↓12%, $p<0.01$). On PND 53, final body weights in the 300 and 1000 mg/kg/day groups were decreased ($p<0.05$) by 7-10%. A treatment-related delay in the mean age at attainment of complete PPS was noted at 1000 mg/kg/day (48.0 days) compared to controls (45.9 days). However, it was determined that the delay in attainment of complete PPS at this dose was a result of the treatment-related decrease in body weight, rather than a direct anti-androgenic effect.

No compound-related effects on organ weights were observed at any dose. No treatment-related effects on T_4, TSH, or testosterone levels were observed at any dose. T_4 and TSH levels were lower than the control group in all treated groups and testosterone was lower at 300 and 1000 mg/kg/day. However, these changes were not statistically significant and were not associated with any histopathological findings. At 1000 mg/kg/day, the increases ($p<0.01$) in ALT (also at

300 mg/kg/day), sodium, albumin, ALP, AST, chloride, phosphorous, and total protein, and the decrease in urea nitrogen were considered to be related to treatment.

At 1000 mg/kg/day, there was a slight increase in the number of animals with colloid area Grade 4 (5 treated vs. 1 control) and Grade 5 (1 treated vs. 0 controls). There were no treatment-related effects on follicular cell height at any dose compared to controls. There were no treatment-related findings in the testes, epididymides or kidneys.

The highest dose tested showed evidence of overt toxicity based on the decreases in terminal body weight, clinical signs, and mortality at 1000 mg/kg/day; the dose concentrations used in the study are considered adequate.

Effects on androgen-related endpoints included decreased ventral prostate, seminal vesicle, LABC, and pituitary glands weights, as well as a 2.1 day delay in PPS at the high dose but these occurred with significant decreases in body weight. There were no treatment-related effects on thyroid-related endpoints in this assay.

Steroidogenesis Assay; (OCSPP 890.1550)

The purpose of this study was to validate the use of a standardized steroidogenesis assay as detailed in *OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 4XX – The H295R Steroidogenesis Assay* (available on-line at http://www.oecd.org/dataoecd/56/11/44285292.pdf). In this validation study, 28 chemicals were selected as a screen for potential effects of endocrine-disrupting chemicals on the production of testosterone (T) and 17β-estradiol (E2). These chemicals were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. These chemicals were selected and approved by the OECD Validation and Management Group for Non-Animal Testing (VMG NA). Glyphosate was one of the chemicals evaluated. A total of seven laboratories from the USA, Denmark, Germany, Japan, Hong Kong, and Canada, each with different levels of experience in conducting the H295R steroidogenesis assay, were invited to participate in this validation study. Inclusion of laboratories with different levels of proficiency in conducting the assay was essential to evaluate the completeness of the test protocols and their transferability. Each laboratory was assigned a random code number (1–7) as part of the study. However, part way through the study, two of the seven laboratories decided to cease their participation in the validation studies. Thus, with the exception of the QC exposure data, only the data for the remaining five laboratories that completed the validation studies are presented (Labs 1, 2, 3, 4, and 6). One laboratory evaluated all 28 chemicals, and one other laboratory (#4) also evaluated glyphosate. The laboratories were not identified.

In this steroidogenesis assay (MRID 48617005), H295R cells cultured *in vitro* in 24-well plates were incubated with glyphosate (purity and lot # not provided) at seven concentrations between 0.0001 and 100 μM (specific concentrations not reported) for 48 hours in triplicate for three independent experiments. The test chemical's vehicle was not identified. The presence or absence of precipitation and/or cytotoxicity was not reported. A Quality Control (QC) plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentrations; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production. T and E2 levels were measured using radioimmunoassays or ELISA; responses of the QC plates measured by these assays were confirmed by LC-MS (at Lab 1).

The report stated that with a few exceptions, all of the laboratories met the key quality performance parameters for conducting the H295R assay protocol. The report stated that two laboratories demonstrated that glyphosate exposure does not affect testosterone or estradiol levels in this assay; however, data were not presented.

Uterotrophic Assay; (OCSPP 890.1600)

In a Uterotrophic Assay (MRID 48617003) conducted to screen for potential estrogenic activity, glyphosate (85.1% a.i., Batch/lot# GLP-1103-21149-T) in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six ovariectomized female Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 (limit dose) mg/kg/day on post-natal days (PND) 66/67 to 68/69. The positive control group was treated with a daily dose of 17α-ethynyl estradiol (EE) at 3 μg/kg/day by oral gavage. Body weights were determined daily. All animals were terminated and necropsied on PND 69/70 approximately 24 hours after the final dose administration to determine wet and blotted uterine weights.

All animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate dosed animals. Body weights, body weight gains, and uterine weights in the glyphosate groups were comparable to the vehicle control.

In the positive control (EE) group, mean body weights decreased on Days 3 and 4 (not significant, NS), leading to an overall body weight loss during the study of 5.6 g ($p<0.01$) compared to a gain of 11.3 g in the controls. Absolute wet and blotted uterus weights for the positive control (EE) group were increased ($p<0.01$) by 758% and 256%, respectively, as expected.

No statistically significant changes were seen in uterine weight in this assay. Glyphosate is negative in the uterotrophic assay.

APPENDIX 2. Other Scientifically Relevant Information (OSRI)

Published literature and Part 158 general toxicity studies considered relevant for determining the potential interaction of glyphosate with the endocrine system are summarized below. Given the breadth of toxicity data available on glyphosate, the only Part 158 studies discussed in this appendix are those that fulfill current guideline requirements and are directly relevant to assessing potential endocrine effects (*i.e.*, multiple-generation rodent reproductive studies, development studies, chronic toxicity studies, etc.).

ER Binding and Transactivation Assays

In a published, non-Guideline study (**Kojima *et al.*, 2004)**, the potential of glyphosate (>95% purity) to interact with two human ER subtypes (hERα and hERβ) and one human androgen receptor (hAR) in transactivation assays with Chinese hamster ovary cells (CHO-K1 cells) was investigated. The human ER (hERα) and AR (hAR) expression vectors (pcDNAERα and pZeoSV2AR, respectively) were constructed as described previously **(Kojima *et al.*, 2003)**. The hERβ expression vector was constructed by cloning the hERβ cDNA with reverse transcriptase-polymerase chain reaction from human placental RNA. The sequence was verified and inserted into the mammalian expression vector pcDNA3.1Zeo(-) creating pcDNAERβ. The construction of the estrogen or androgen responsive elements containing reporter plasmid (pGL3-tkERE and pIND-ARE, respectively) was described previously **(Kojima *et al.*, 2003)**. pRL-SV40 containing the *Renilla* luciferase gene was used as an internal control for transfection efficiency.

For detection of hERα or hERβ activity, cells were transfected with pcDNAERα or pcDNAERβ, pGL3-tkERE, and pRL-SV40. For detection of hAR activity, cells were transfected with pZeoSV2AR, pIND-ARE, and pRL-SV40. Flutolanil was tested over a concentration range from 10^{-8} to 10^{-5} M, and the responses were compared to the activity of 17β-estradiol (E_2, >97% purity) or 5α-dihydrotestosterone (DHT, 95% purity) for the ER or AR assays, respectively. For measurement of antagonistic activity to hERα, hERβ and hAR, either 10^{-11} or 10^{-10} M E_2 or 10^{-10} M DHT was added to the cell cultures along with the test compound, respectively. Agonistic activities were evaluated by the relative activity expressed as 20% relative effective concentration (REC_{20}), the concentration of the compound demonstrating 20% of the activity of 10^{-10} M E_2, 10^{-9} M E_2, or 10^{-9} M DHT for ERα, ERβ, and AR, respectively. Antagonistic activities were evaluated similarly by the relative activity expressed as 20% relative inhibitory concentration (RIC_{20}), the concentration of the compound showing 20% inhibition of the activity of E_2 or DHT against ERα, ERβ, and AR, respectively. Glyphosate did not have agonist or antagonist activity for ERα and ERβ, or agonist activity for AR in this study.

Thongprakaisang *et al.* (**2013**) evaluated the estrogenic and/or anti-estrogenic effects of glyphosate (>98% purity) at concentrations that have been reported in environmental conditions and exposed humans. Effects were investigated in estrogen-dependent human breast cancer cells, T47D; a stably ERE (estrogen response element)-luc construct-transfected, hormone-dependent

breast cancer, T47D-KBluc; and a hormone-independent human breast cancer, MDA-MB231. To differentiate the effects of endogenous estrogen, cells were studied both in completed (standard) medium (medium containing fetal bovine serum) and in estrogen withdrawal medium (medium containing dextran-charcoal treated fetal bovine serum).

Cells were seeded in 96-well luminometer plates and allowed to attach overnight. Media was then replaced with dosing media in which the final concentration of glyphosate ranged from 10^{-12} to 10^{-6} M. 17β-estradiol (E2) at the same range of concentrations was used as the positive control agonist for ER activation. The media was used as the negative control. Cells were allowed to incubate 24 hours. Cell growth and viability were tested with the MTT reagent assay.

In the cell viability assay, glyphosate caused hormone-dependent T47D cells to proliferate, approximately 15-30% of control, in the absence of E2; the effect was significant ($p \leq 0.05$) at 10^{-9} and 10^{-8} M and was approximately half the E2 response in T47D cells. Glyphosate had no effect on the growth of hormone-independent MDA-MB231 cells in the absence or presence of E2. Because the proliferation effect of glyphosate on T47D cells was only observed in the absence of E2, the possibility that ER signaling may be involved was investigated by co-incubating glyphosate or E2 with the ER antagonist, ICI 182780, in T47D cells. ICI 182780 at a concentration of 1 nM mitigated the proliferative effect of glyphosate and E2 in T47D cells; the proliferative effects of glyphosate on T47D cells were completely inhibited at an ICI 182780 concentration of 10 nM. The results suggest that the proliferative effects of glyphosate may involve the ER.

The estrogenic activity of glyphosate on ERE transcription activity was investigated with T47D-KBluc cells. In the concentration range 10^{-12} to 10^{-6} M, glyphosate induced ERE activation 5- to 13-fold compared to control; the effects were <50% compared to E2 induction. Co-incubation of glyphosate with 1 nM ICI 182780 resulted in a decreased response, with complete inhibition in the presence of 10 nM ICI 182780. When cells were co-incubated with glyphosate and E2, the E2-induced ERE activation was suppressed, indicating that in the presence of endogenous agonist (E2), glyphosate acted as an antagonist.

90-Day (Subchronic) Oral Toxicity in Rodents (Rat; OSCPP 870.3100)

In a subchronic oral toxicity study in rats (**MRID 40559401**), glyphosate (95.21% purity) was administered to SD rats (12/sex/group) in the diet at nominal concentrations of 0, 1000, 5000, or 20,000 ppm for three months. Mean actual intakes were 950, 4600, and 19,000 ppm corresponding to 63, 317, and 1267 mg/kg/day for males and 84, 404, and 1623 mg/kg/day for females (based on actual body weights, food consumption, and analyses of feed). Body weights and food consumption were measured weekly, and hematology, clinical chemistry, and urine analyses were conducted at the end of the study. Organs that were weighed included the liver, kidneys, and testes (including epididymides). For control and high-dose animals, gross and microscopic pathological examinations were conducted on these tissues as well as adrenals,

ovaries, mammary gland, prostate, pituitary, seminal vesicles, thyroid, urinary bladder, and uterus (corpus and cervix). The kidneys and livers of the low- and medium-dose animals were also examined microscopically.

There were no mortalities or treatment-related toxic signs during the study, and there were no effects of treatment on body weight or food consumption. Possibly treatment-related findings in male rats were increased ($p<0.05$) serum phosphorus (low and high dose) and potassium (medium dose) concentrations; increased serum glucose concentrations at 317 and 1,267 mg/kg/day at the medium and high doses; and increased (NS) serum BUN and alkaline phosphatase concentrations at the high dose apparently due to a single animal. In addition, histopathological lesions observed in the pancreas of high-dose male rats may have been treatment-related. Possible treatment-related findings in female rats were increased ($p<0.05$) serum phosphorus concentrations and increased ($p<0.05$ or NS) potassium concentrations in all treated females. No other histopathological findings were noted. Because clinical chemistries were conducted only once during the entire study, and as the pancreas was not examined histologically in the low- and medium-dose groups, it could not be determined whether or not these findings were attributable to the test material on the basis of this study alone.

In a subchronic oral toxicity study in rats conducted by the **National Toxicology Program** (**NTP; 1992**), glyphosate (99% purity) was administered in the diet to F344/N rats (10/sex/group) at nominal concentrations of 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm (mean-measured doses of 205, 410, 811, 1678, or 3393 mg/kg/day for males and 213, 421, 844, 1690 or 3393 mg/kg/day for females, respectively, based on food consumption and body weight measurements) for 90 days. Ten additional rats/sex were included at each dose level for evaluation of hematology and clinical chemistry parameters. Body weights were measured weekly. Samples for hematology and clinical chemistry determinations were collected from additional study animals on Study Days 5 and 21, and from the regular study animals at the end of the study. Organs that were weighed included kidney (right), liver, and testis (including left caudal and left epididymal tissue for the control and 12,500, 25,000, and 50,000 ppm dose groups). For control and high dose animals, gross and microscopic pathological examinations were conducted on these tissues as well as adrenals, mammary gland, ovaries, pituitary, preputial/clitoral glands, prostate, seminal vesicles, thyroid, uterus, and vagina; histopathological examinations of the salivary gland were conducted for rats in all dose groups. In addition, for rats in the control and three highest dose groups, sperm morphology (sperm motility, sperm count per gram caudal tissue, and spermatid head count) evaluations were conducted at the end of the study, and vaginal cytology evaluations were conducted during the 12 days prior to sacrifice.

All animals survived to necropsy. Diarrhea was noted in the high-dose animals (males and females) through Day 50 of the study. Body weight gain was reduced in the high-dose males (↓25%; 3393 mg/kg/day) and the final body weight was decreased by 18%, but statistical significance was not reported for final body weights or body weight gains. Mean necropsy body

weights were decreased ($p<0.05$ and 0.01, respectively) for the 1678 and 3393 mg/kg/day males (↓12 and 21%, respectively). Body weight gain in high-dose females (3393 mg/kg/day) was decreased (↓10%), but no effects on final or necropsy body weights were observed. Clinical chemistry results showed increases in ALP and ALT in some or all of the dose groups; ALP concentrations in the control animals was variable over the sampling days (Days 5, 21, and 90). Increases ($p<0.01$) in ALP in males were generally dose-dependent in the top three dose groups on Days 5 and 21, but were similar to control by Day 90. Increases ($p<0.01$ or 0.05) in ALP in females were dose-dependent in the top three dose groups on Days 5 and 21, and still present on Day 90 in the top two dose groups. Increases in ALT in both sexes were generally non-dose-dependent, and seen in all but the lowest dose group in males (except for Day 90) and in all dose groups in females (except for Day 90). In addition, effects on bile acids were noted in both sexes. On Day 5, variable increases (NS) were noted with a clear dose-dependent response in the two highest dose groups. A stronger, dose-dependent response was observed on Day 21 with increases (NS, $p<0.05$, and $p<0.01$) in males (↑2-70%) and females (↑23-102%). Concentrations of bile acids on Day 90 were more variable, with a generally non-significant, but dose-dependent response at the two highest doses in both sexes. Non-dose-dependent increases ($p<0.01$ or 0.05) in relative liver weights also were observed in males in all dose groups (↑6-15%) and in females in the two highest dose groups (↑10 and 6%). Taken together, these findings are consistent with a hepatobiliary effect that can be attributed to glyphosate administration.

In the 1678 and 3393 mg/kg/day males, there were increases ($p<0.01$ or 0.05) in the relative right kidney (↑8% and 13%, respectively) and right testis weights (↑7% and 21%, respectively); relative right kidney weight also was increased ($p<0.05$) in high-dose females (↑7%). Absolute weights for these organs in treated rats were similar to control. Left caudal, epididymal, and testicular weights of treated males (811, 1678, and 3393 mg/kg/day groups) were similar to control.

A dose-related increase in the incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands was noted in the microscopic evaluations, beginning at the lowest dose in both sexes. These lesions were diagnosed as “cytoplasmic alteration” and consisted of basophilic change and hypertrophy of acinar cells. No other treatment-related histopathological findings were reported.

Sperm counts were decreased ($p<0.01$) in the two high-dose groups (1678 and 3393 mg/kg/day; ↓20%). Epididymal sperm motility, total spermatid head/testis, and total spermatid heads/g caudal tissue in the treated animals were similar to control. A delayed ($p<0.05$) estrous cycle was observed in the 3393 mg/kg/day group (5.4±0.21 days) compared to control (4.9±0.10 days).

The LOAEL is the low dose (205/213 mg/kg/day for males and females, respectively) based on dose-related increases in the incidence and severity of cytoplasmic alterations in the parotid and submandibular salivary glands of male and female rats. A NOAEL is not determined.

90-Day (Subchronic) Oral Toxicity in Rodents (Mouse; OSCPP 870.3100)

In a subchronic oral toxicity study in mice (**MRID 00036803**), glyphosate (98.7% purity) was administered to CD-1 albino mice (15/sex/group) at dietary concentrations of 0, 5000, 10,000, or 50,000 ppm (0, 750, 1500, or 7500 mg/kg/day, based on standard conversion) for three months. The mice were sacrificed at the end of the study and complete gross necropsies were conducted. Organs that were weighed included the gonads, kidneys, and liver. Histopathological examinations included these organs, as well as adrenals, epididymis, pituitary, thyroid, parathyroid, urinary bladder, uterus, and prostate.

There were no treatment-related toxicological signs or mortalities during the study, and there were no effects of treatment on food consumption. Body weight gains in the male and female high-dose groups were decreased (↓ approximately 24% and 18%, respectively) compared to control. There were no changes in absolute or relative organ weights at any dose, and there were no treatment-related histopathological findings in any organ. The LOAEL for the study is 50,000 ppm (7500 mg/kg/day) based on reduced body weight gain and the NOAEL is 10,000 ppm (1500 mg/kg/day).

In a subchronic oral toxicity study in mice conducted by **NTP (1992**), glyphosate (99% purity) was administered in the diet to B6C3F1mice (10/sex/group) at nominal concentrations of 0, 3125, 6250, 12,500, 25,000, or 50,000 ppm (equivalent to 0, 507, 1065, 2273, 4776, or 10,780 mg/kg/day for males and 753, 1411, 2707, 5846, 11,977 mg/kg/day for females, respectively, based on food consumption and body weight measurements) for 90 days. Body weights were measured weekly. Organs that were weighed included kidney (right), liver, and testis (including left caudal and left epididymal tissue for the control and 12,500, 25,000, and 50,000 ppm dose groups). For control and high-dose animals, gross and microscopic pathological examinations were conducted on these tissues as well as adrenals, mammary gland, ovaries, pituitary, preputial/clitoral glands, prostate, seminal vesicles, thyroid, uterus, and vagina; histopathological examinations of the salivary gland were conducted for mice in all dose groups. In addition, for mice in the control and three highest dose groups, sperm morphology (sperm motility, sperm count per gram caudal tissue, and spermatid head count) evaluations were conducted at the end of the study, and vaginal cytology evaluations were conducted during the 12 days prior to sacrifice.

There were two mortalities in the study; neither was related to treatment. In addition, no clinical signs of toxicity were reported. Final body weights were decreased in males and females in the two highest dose groups (↓11% and 17% for males and 6% and 10% for females) with concomitant decreases in body weight gain (↓35% and 63% for males and 14% and 27% for females); statistical significance was not reported. Mean necropsy body weights were decreased for the 4776/5846 mg/kg/day ($p<0.05$; ↓8 and 6%, respectively) and 10,780/11,977 mg/kg/day

($p<0.01$; ↓15 and 11%) males and females. Average daily food consumption was comparable across the treated and control groups.

Relative right testis weights were increased ($p<0.01$) at the two highest doses (↑10% at 4776 mg/kg/day and ↑18% at 10,780 mg/kg/day); absolute weights were comparable to control. Absolute left caudal, epididymal, and testicular weights of treated males (2273, 4776, and 10,780 mg/kg/day groups) were similar to control. The relative weights of the right kidney were increased ($p<0.05$), in a non-dose-related manner, in males in all dose groups except the lowest (↑11%, 15%, 23%, and 16%, respectively); absolute kidney weights were increased only in the medium-dose males (↑15%). Non-dose-dependent increases ($p<0.05$) in relative liver weight also were noted in males of all dose groups except the lowest (↑9%, 4%, 8%, and 9%, respectively). A decrease ($p<0.01$) in the absolute liver weight was noted in high-dose females (↓14%).

Dose-related increases in the incidence and severity of lesions in the parotid salivary glands of male and female mice were observed, beginning at the second lowest dose (1065 and 1411 mg/kg/day, respectively); no lesions were observed in mice in the low-dose group. These lesions were diagnosed as "cytoplasmic alteration" and consisted of basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. This finding was considered adverse.

Histopathology examination of control and high dose animals did not reveal any other related findings. There were no effects of treatment on sperm counts, sperm motility, total spermatid head/testis, and total spermatid heads/g caudal tissue in males, or on length of estrous cycle in females.

Prenatal in Rodents (Rat; OCSPP 870.3700)

In a developmental toxicity study in rats (**MRID 00046362**), glyphosate (98.7%) was administered by gavage to mated female SD rats (25/group) at doses of 0 (0.5% aqueous methocel), 300, 1000, or 3500 mg/kg/day on GDs 6 to 19, inclusive. Individual dam body weights were recorded on GDs 0, 6, 9, 12, 16, and 20; food consumption was not determined. The surviving rats were sacrificed on GD 20 and the uterus was weighed; the locations of viable and nonviable fetuses, early and late resorptions, and the total number of implantations and corpora lutea were recorded. All fetuses were weighed, sexed, and examined for gross external malformation. One-half of the fetuses in each litter were examined for visceral soft tissue alterations by the Wilson technique. The remaining fetuses in each litter were examined for skeletal alterations by the Dawson method. This study was conducted prior to the publication of EPA GLP Guidelines.

There were six deaths in the high-dose group (one each on GDs 10 and 17, and two each on GDs 11 and 12); the causes of death were not determined. At the high-dose, all of the dams (except three) were observed at least once with diarrhea, soft stools, breathing rattles, inactivity, and red

matter in the region of nose, mouth, forelimbs, or dorsal head; no treatment-related clinical signs were observed in the low- and mid-dose groups. There were no treatment-related effects on maternal body weight in the low- and mid-dose groups. At the high dose, maternal body weight gain was decreased (↓23%) during the test period (GDs 0-20), due to a body weight losses during GDs 6-20 (↓6-10%). It is not known if statistical analyses were conducted on body weights/body weight gains, as no statistical differences were reported for these decreases.

At 300 and 1000 mg/kg/day, there were no effects in mean number of viable fetuses, late or early resorptions, postimplantation loss, corpora lutea, the fetal sex distribution, or fetal body weight. Decreases in viable fetuses/dam (11.9 ± 4.36; $p<0.05$) and total implantations/dam (12.1 ± 4.45; $p<0.01$) were noted at the low dose compared to control (14.4 ± 1.26 and 15.0 ± 1.67, respectively), but were not considered to be related to treatment because the decreases were not dose-related. At the high dose, decreases in viable fetuses/dam (11.5 ± 4.12; $p<0.05$), total implantations/dam (12.8 ± 3.77; $p<0.05$), and mean fetal body weight (3.2 ± 0.34 g vs. 3.0 ± 0.21 g in control; $p<0.01$) were observed. There were no fetal malformations in low- and mid-dose groups. The number of malformed fetuses in the high-dose group (10) was increased compared to control (3). However, because the number of litters with malformed fetuses was the same for both groups, this was not considered to be an effect. There was an increase in the number of litters and fetuses with unossified sternebrae observed at the high dose. The maternal LOAEL is 3500 mg/kg/day, based on mortality, toxic signs, and decreased body weight gain, and the maternal NOAEL is 1000 mg/kg/day. The developmental LOAEL is 3500 mg/kg/day based on decreases in fetal body weight, increases in the number of litters and fetuses with unossified sternebrae, decreases in viable fetuses/dam, and decreases in total implantations/dam; the developmental NOAEL is 1000 mg/kg/day.

Prenatal Development in Non-Rodents (Rabbit; OCSPP 870.3700)

In a developmental toxicity study in rabbits (**MRID 00046363**), glyphosate (98.7% purity) was administered to artificially-inseminated, female Dutch Belted rabbits (16/dose group) by gavage at doses of 0 (0.5% aqueous methocel), 75, 175, or 350 mg/kg/day from GDs 6-27, inclusive. Individual doe body weights were recorded on GDs 0, 6, 12, 18, 24, and 28; food consumption was not determined. On GD 28, all surviving does were sacrificed, and the uterus was examined and weighed; the number and location of viable fetuses and early and late resorptions, and the total number of implantations and corpora lutea were recorded. Each fetus was weighed, examined for external malformations, dissected, sexed, examined for visceral abnormalities, then eviscerated, stained with Alizarin Red S, and examined for skeletal abnormalities by the Dawson method. This study was conducted prior to the publication of EPA GLP Guidelines.

One rabbit in the low-dose group died apparently of pneumonia on GD 26. Two rabbits in the control group, one in the low-dose group, and one in the high-dose group aborted and were sacrificed. Additionally, two rabbits at the medium dose and ten rabbits at the high dose died

during the study; of these, death was attributed to gastroenteritis for two rabbits, to enteritis for one rabbit, and to respiratory disease for one rabbit. The cause of death for the remaining eight rabbits could not be determined at necropsy. Soft stool or diarrhea was noted in all groups with a slight increase at the medium dose and at least once in each rabbit of the high-dose group; in addition, an increase in nasal discharge was noted in the high-dose animals. There were no toxicologically significant differences in maternal mean body weight among control and treated groups; however, a non-dose-dependent decrease in overall body weight gain was observed in the medium-dose group (↓135%). No treatment-related changes were seen in the number of viable fetuses, early or late resorptions, total implantations, corpora lutea, or fetal sex distributions at any dose. A slight decrease was noted for mean fetal body weight of all of the treated groups compared to the control group. However, mean fetal body weights for all groups were comparable to historical control mean fetal body weight values. There were no treatment-related malformations in fetuses from litters of treated rabbits in comparison to control. A few malformations were noted in each of the three treated groups. Because these did not occur in a dose-related pattern and their frequency was comparable to that of the historical control group, they were not considered treatment-related.

The maternal LOAEL is 350 mg/kg/day, based on increased incidences of soft stool, diarrhea, nasal discharge, and death, and the maternal NOAEL is 175 mg/kg/day. The developmental LOAEL is not determined (>350 mg/kg/day), and the developmental NOAEL is 350 mg/kg/day.

Multi-Generation Reproduction in Rodents (Rat; OCSPP 370.3800)

In a three-generation reproduction study in rats (**MRID 00081674**), glyphosate (98.7% purity; assumed to be 100% for dosing preparations) was administered in the diet to three consecutive generations of CD (SD) rats (12 males and 24 females/generation/dose) at nominal doses of 0, 3, 10, or 30 mg/kg/day. Each parent generation was mated to produce two litters. Offspring from the second litters of the F_0 and F_1 parents (F_{1b} and F_{2b} litters, respectively) were selected to be parents for the subsequent pair of generations. Parameters evaluated for each generation included: mortality, body weight and food consumption data, in-life physical observation data, maternal body weights (gestation/lactation), reproduction-fertility indices (mating, pregnancy and fertility indices), litter data at parturition, and organ weight data. Offspring from each litter interval were evaluated during a 21-day lactation period for growth, survival, sex distribution data, and gross postmortem observations including organ weight data (F_{3b} offspring only). All parents were sacrificed after weaning of the second litters and randomly selected offspring from the second litters of the second generation (21-day F_{3b} weanlings) were sacrificed; the organs that were weighed at sacrifice included the adrenals, gonads, kidneys, liver, and pituitary. Microscopic examinations of these tissues as well as mammary glands, thyroid and parathyroid, prostate, and uterus were conducted for 10 rats/sex from the control and high-dose groups of F_0, F_1, and F_2 parents and F_{3b} weanlings.

There were no treatment-related effects on adult mortality, clinical signs, body weight, or food consumption. Although there were some differences between control and treated groups, there were no consistent, dose-related effects on mating, fertility, or pregnancy indices over the entire study that would indicate an adverse effect of treatment. There were no consistent adverse effects of treatment in maternal body weight or body weight gain, gestation length, parturition data, or litter survival indices throughout the study. No consistent treatment-related effects were found in sex distribution data, body weights, survival, or gross postmortem findings in offspring. There were no effects of treatment on absolute or relative organ weights in F_{3b} offspring or F_0, F_1, or F_2 adult males. Gross postmortem evaluations of the tissues from randomly selected F_0, F_1, and F_2 control and high-dose animals, and F_{3b} control and high-dose offspring, revealed a high incidence of focal tubular dilation in the kidneys of the male F_{3b} high-dose offspring. In a subsequent submission (**MRID 00105995**), the results of examination of the kidneys from the low- and mid-dose F_{3b} offspring were reported, which showed that the incidence of renal focal tubular dilation in the low- and mid-dose F_{3b} offspring was similar to the study control, as well as, historical control data.

The reproductive LOAEL is 30 mg/kg/day based on increased focal tubular dilation in the kidneys of male F_{3b} offspring. The reproductive NOAEL is 10 mg/kg/day. The systemic NOAEL is 30 mg/kg/day, and the LOAEL is not determined (>30 mg/kg/day).

In a two-generation reproduction study with rats (**MRID 41621501**), glyphosate (97.67% purity) was administered in the diet to SD rats (30/sex/group) at 0, 2000, 10,000, or 30,000 ppm (0, 100, 500, or 1500 mg/kg/day, based on standard conversion) for eleven weeks. After Week 11, the rats were mated to produce the F_1 generation. Litters were culled to eight pups and weaned on Day 21, after which 30 rats/sex/group were randomly selected to continue as the second generation. The F_1 rats were mated after approximately 14 weeks to produce the F_2a and F_2b litters. Rats were maintained on dosing diets throughout pre-mating, mating, gestation, and lactation until sacrifice. Parameters evaluated for each generation included: mortality, clinical signs, body weight, and food consumption, maternal body weights (gestation/lactation), reproduction-fertility indices (pre-coital interval, gestation length, litter size, dead pups/litter, and pup survival), and organ weight (ovaries and testes with epididymides). Microscopic examinations of tissues of F_0 and F_1 adults were conducted for selected tissues: kidneys, ovaries, pituitary (F_1 only), prostate, seminal vesicles, mammary gland, testes, epididymides, and uterus/vagina. In addition, kidneys from F_2b weanlings (1/sex/litter) were examined microscopically.

There were no treatment-related mortalities during the study. Treatment-related clinical signs consisted of soft stools in high-dose males and females (both F_0 and F_1). Body weights of high-dose male and female F_0 rats were decreased ($p<0.01$, ↓8%) during the premating growth period. Similarly, F_1 high-dose male and female adults weighed less at weaning compared to F_0 animals with continued decreases ($p<0.01$ or 0.05) of 10-12%. The body weights of high-dose females

remained decreased ($p<0.01$ or 0.05) compared to control during gestation and lactation of the F_1, F_2a, and F_2b litters, although high-dose body weights were comparable to control by the end of lactation. Food consumption was generally similar to control in high-dose males and females during most measurement periods; consumption was decreased in the medium- and high-dose F_1 females on Days 0-14 (↓9 and 11%, respectively). There were no treatment-related effects on mating and fertility in the F_0 to F_1 generation, or in the F_1a to F_2a or F_1a to F_2b generations. At Days 14 and 21 in the F_2b litter, and Day 21 in F_1 and F_2a litters, the mean body weights of high-dose pups were decreased ($p<0.01$ or 0.05, ↓6-14%) and body weight gain of high-dose pups was decreased 15-20%. There were no consistent, dose-related effects on litter size at Day 0 or on pup survival during lactation. There were no treatment-related findings in gross necropsy or histopathology in examined treated rats compared to control. Terminal body weights in the F_0 and F_1a generations were decreased ($p<0.01$) by 8-13%. Increased relative weights of testes of high-dose F_0 (NS, ↑6%) and F_1 ($p<0.01$, ↑12%) rats were noted; however, this effect was considered to be due to the decreased terminal weights, and not be of toxicological significance as absolute weights were not affected by treatment. There were no treatment-related gross or microscopic findings in the kidneys of F_{2b} weanlings.

The reproductive NOAEL is 30,000 ppm (1500 mg/kg/day). The systemic LOAEL is 30,000 ppm based on increased incidences of soft stool, and decreased food consumption and body weight during growth; the systemic NOAEL is 10,000 ppm (500 mg/kg/day). The developmental LOAEL is 30,000 ppm based on decreased pup body weight on LDs 14 and 21; the developmental NOAEL is 10,000 ppm.

In a subsequent two-generation reproduction study with rats (**MRIDs 48865101 through 48865105**), glyphosate (95.7% purity) was administered to Crl:CD(SD) IGS BR rats (28/sex/dose) in the diet at nominal doses of 0, 1500, 5000 and 15,000 ppm (equivalent to 121/126, 408/423, and 1234/1273 mg/kg/day in males/females during pre-mating) for two successive generations with one litter per generation. The P generation animals were fed the test diets for ten weeks prior to mating to produce the F_1 litters. The F_1 litters were not standardized; litter sizes ranged from 4 to 21 pups. On PND 21, 24 pups/sex/dose were selected and fed the same test diet as their parents for ten weeks prior to mating to produce the F_2 litters. Organs that were weighed in parental animals included adrenals, epididymides, kidneys, liver, ovaries, prostate, pituitary, seminal vesicles, testes, thymus, thyroid, and uterus; of these tissues, adrenals, epididymides, ovaries, prostate, pituitary, seminal vesicles, testes, and uterus of the control and high-dose P and F_1 group were subjected to microscopic examination.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, or gross or microscopic pathology in either the P or F_1 generation.

Absolute and relative (to body) liver weights were increased ($p<0.05$, 0.01, or 0.001) by 8-13% in both the P and F1 females at in the high-dose group with no histopathological findings. Additionally, at this dose, absolute and relative kidney weights were increased ($p<0.01$ or 0.001) by 7-11% in the P females only.

The LOAEL for parental toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

There were no effects of treatment on the mean numbers of corpora lutea and implantations, pre- and post-implantation loss, numbers of pups born, litter size on PND 1, 4, 7, 14, and 21, live birth, viability, and lactation indices, and sex ratio at birth and on PND 1 and 21, clinical signs of toxicity, litter weights, pup body weights, developmental landmarks or reflexes, ano-genital distance, or brain, spleen or thymus weights in either the F1 or F2 offspring.

There was a delay ($p<0.01$) of 2.9 days in attaining complete PPS (PND 45.9 treated vs. PND 43.0 control) in the 15,000 ppm F1 male pups, along with a 10% increase ($p<0.01$) in body weight at attainment.

The LOAEL for offspring toxicity is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating), based on delayed age and increased weight at attainment of PPS. The NOAEL is 5000 ppm (equivalent to 408/423 in males/females during pre-mating).

The number of males impregnating their female partner, and the number of females not pregnant, with no litters observed, with total litter loss, and rearing their litters to weaning was similar in all groups. The pre-coital intervals were similar across all groups with the majority of pairs mating during the first four days of the mating period. Gestation duration, mean estrous cycle length and periodicity, follicle number, and sperm parameters were unaffected by treatment.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

Chronic Oral Toxicity/Carcinogenicity in Rodents (Rat; OCSPP 870.4300)

In a chronic oral toxicity/carcinogenicity study in rats (**MRIDs 00093879 and 00150564**), glyphosate (98.7% purity) was administered to rats (50/sex/group) at nominal concentrations of 0, 30, 100, or 300 ppm (3, 10, or 30 mg/kg/day based on standard conversion) for the first week of the study; rats were then dosed at 3.05, 10.30 and 31.49 mg/kg/day for the males and 3.37, 11.22 and 34.02 mg/kg/day for the females for the remainder of the study. Animals were terminated at 26 months, when survival had decreased to 30% in one group per sex. All rats were observed twice daily for mortality and toxic signs and given a detailed physical examination each week throughout the study. Body weights and food consumption were determined at pretest, weekly through 14 weeks, and biweekly for the remainder of the study. Clinical and

hematological parameters were determined (10 rats/sex/group) at 4, 8, 12, 18, and 24 months. Complete necropsies were performed on all rats that died or were sacrificed during or at the end of the study. Organ weights determined at sacrifice included the adrenals, kidneys, liver, testes/ovaries, pituitary, and thyroid. Microscopic examination was conducted on these tissues, as well as epididymides, mammary gland, parathyroid, prostate, seminal vesicles, urinary bladder, and uterus for all treated and control rats.

Survival was unaffected by treatment. Decreased (NS) body weight gains were observed in treated males beginning at Week 26 and continuing to Week 102, and decreased (significance not reported) body weight gains (↓10-15%) were present in the low- and medium-dose female rats from Weeks 84 to 92. No dose-response relationship was present in the decreased body weight gains in female rats and the changes in body weight did not affect survival. Overall food consumption was unaffected by treatment. Hematology, clinical chemistry, and urinalyses values for treated male and female rats were comparable to control values. Absolute and relative organ weights of treated rats also were comparable to control. No treatment-related findings were observed during gross necropsies. Microscopic examinations revealed lymphocytic hyperplasia of the thymus occurring at increased (significance not reported) incidences in the mid- and high-dose female rats. In addition, focal vacuolation of liver was found with increased (significance not reported) incidence in high-dose male rats. Testicular interstitial cell tumors were seen in 3/50, 1/50 and 6/50 rats at the low-, mid-, and high-dose groups when compared to control (0/50) with the 12% incidence at the high dose reaching statistical significance ($p=0.013$). However, it was concluded that the increased incidence was similar to the incidence observed in control groups of male rats in other concurrent studies and therefore was not related to treatment. C-cell carcinomas were seen in the thyroid of 5/47 females at the high dose (11%; $p<0.001$) compared to 1/47 control females (2%). No increases were seen in adenomas, and the incidences of C-cell hyperplasia were comparable between the treated and control groups.

The LOAEL for chronic toxicity is 10.30/11.22 mg/kg/day (males/females) based on increased lymphocytic hyperplasia. The NOAEL for chronic toxicity is 3.05/3.37 mg/kg/day (males/females). The oncogenic potential is negative.

In a subsequent chronic oral toxicity/carcinogenicity study in rats (**MRID 41643801**), glyphosate (96.5% purity) was administered to SD rats (60/sex/group) at nominal concentrations of 0, 2000, 8000, or 20,000 ppm (89, 362, and 940 mg/kg/day in males and 113, 457, and 1183 mg/kg/day in females) for 24 months; 10 rats/sex/group were assigned for interim sacrifice after 12 months of treatment. All rats were observed twice daily for mortality and toxic signs and given a detailed physical examination each week throughout the study. Body weights were determined once weekly for 13 weeks and then monthly thereafter. Ophthalmological examinations were conducted pretest and twice prior to sacrifice. Clinical and hematological parameters were determined (10 rats/sex/group) before treatment and at 6, 12, 18, and 24 months; urinalyses also were conducted at 6, 12, 18, and 24 months. The organs that were weighed at sacrifice included

liver, kidneys, testes, epididymides, and prostate. Gross and microscopic pathological examinations were conducted on these and other tissues, including the seminal vesicles, ovaries, uterus, urinary bladder, mammary glands, pituitary, adrenals, and parathyroids/thyroids.

There were no treatment-related effects on clinical signs or mortality. The mean body weight of high-dose females was decreased ($p<0.05$) by 3% at Day 51, 13% ($p<0.01$) at Week 81, and 3% (NS) at Week 104; at Week 81, body weight gain was decreased by 23% in high-dose females compared to control. There were no decreases in body weight or body weight gain in male rats. The incidence of cataracts and lens abnormalities in male rats in the high-dose group was increased ($p<0.05$) compared to control according to two examiners, and not significantly different according to two other examiners. There were no treatment-related hematology or clinical chemistry findings that were considered toxicologically significant.

Liver weight (relative to body weight) was increased ($p<0.05$, ↑13%) in high-dose males at interim sacrifice, and absolute liver weights were increased ($p<0.05$, ↑13%) in high-dose males at terminal sacrifice. No treatment-related changes were seen in absolute or relative weights of the testes, epididymides, and prostate glands, and no treatment-related histopathological lesions were observed in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, and mammary, adrenal, and pituitary glands. The incidences of the pancreatic islet call adenomas in the low-, medium- and high-dose males exceeded the historical control range (1.8-8.5%); however, because there was no positive dose-related trend in the occurrence of these tumors in males, no progression to carcinoma, and the incidence of hyperplasia was not dose-related, the pancreatic islet cell tumors were not considered to be treatment-related. The incidences of thyroid C-cell adenomas were increased in animals in the medium- and high-dose groups compared to control; however, these increases were not considered to be treatment-related because none of the increases reached statistical significance, there was no dose-response in either sex, there was no increase in severity of grade or incidence in hyperplasia or progression to malignancy, and the incidences were within the historical control ranges.

The NOAEL is 8000 ppm (362 and 467 mg/kg/day in males and females, respectively) and the LOAEL is 20,000 ppm (940 and 1183 mg/kg/day in males and females, respectively) due to decreased body weight and body weight gain in females, cataracts in males, and increased relative liver weight (to body) at 12 months and increased absolute liver weight at 24 months in males.

In addition, the HED Carcinogenicity Peer Review Committee evaluated the carcinogenic potential of glyphosate in June 1991. Glyphosate was classified into Group E (evidence of non-carcinogenicity in humans) based on a lack of convincing evidence of carcinogenicity in adequate studies in two species.

Carcinogenicity in Rodents (Mouse; OCSPP 870.4200)

In a carcinogenicity study in mice (**MRIDs 00130406 and 00150564**), glyphosate (99.7% purity) was administered in the diet to CD-1 mice (50/sex/group) at nominal concentrations of 0, 1000, 5000, or 30,000 ppm (0, 150, 750, or 4500 mg/kg/day, based on standard conversion) for 24 months. The parameters evaluated were clinical signs, mortality, body weight, food and water consumption, and hematology at 12, 18, and 24 months. At the end of the study, all animals were necropsied and selected organs (not specified) were weighed; tissues (not specified) were stained and examined microscopically.

There were no treatment-related toxic signs noted, and mortality was low during the first eighteen months of the study. Body weight was consistently decreased for males and, to a lesser extent, females in the 30,000 ppm dose group during the study at several sampling intervals (extent and significance not reported). Changes in body weight at the low- and mid-dose group were variable and not dose-related. Food consumption showed no treatment-related or dose-related effect. Hematological values, although significant in some instances, did not show a consistent dose-related response. Necropsy did not show treatment-related lesions. There was good correlation between gross and microscopic findings. The relative (to bodyweight) testes weight was non-significantly increased (17.5%) at the high dose; however there were no significant changes in absolute or relative-to-brain weights for the testes. Absolute and relative ovarian weights were increased at 30000 ppm; however, the change was not statistically significant and attributed to a single animal in the group. There were no histopathological findings in the ovaries.

Renal tubule adenomas were found in male mice in the 5000 and 30,000 ppm dose groups; however, it was concluded that the renal tumors were not dose-related. Trends, and effects in the high-dose group ($p<0.01$), were observed in non-neoplastic lesions. The lesions considered treatment-related were hepatocyte hypertrophy, central lobular hepatocyte necrosis, and chronic interstitial nephritis in high-dose males, and proximal tubule epithelial basophilia and hypertrophy in high-dose females.

The NOAEL for systemic effects was 5000 ppm (750 mg/kg/day). At the LOAEL, 30,000 ppm (4500 mg/kg/day), there were increased incidences of hepatocyte hypertrophy, hepatocyte necrosis, and interstitial nephritis in male mice and increased incidences of proximal tubule epithelial basophilia and hypertrophy in female mice. Additionally, there were treatment-related decreases in body weights in male and female mice at 30,000 ppm.

Chronic Oral Toxicity in Non-Rodents (Dog; OCSPP 870.4100)

In a chronic toxicity study in dogs (**MRID 00153374**), glyphosate (96.13% purity) was administered to Beagle dogs (6/sex/group) at concentrations of 0, 20, 100, or 500 mg/kg/day for one year. Criteria evaluated included clinical signs, mortality, body weight, food consumption,

ophthalmologic evaluation, hematology, clinical chemistry, urinalysis, organ weights (including adrenals, kidneys, liver, ovaries, testes, pituitary, and thyroid/parathyroid), and histopathology of selected tissues (not reported) and all gross lesions.

All animals survived the duration of the study. There were no dose-related, clinical signs of toxicity, and body weight and food consumption in treated dogs were comparable to control. There were no dose-related changes in hematological or clinical chemistry values. Decreased pituitary weights (absolute and relative) were observed in mid-and high-dose male dogs, however, these were not considered to be treatment-related because there were no underlying histopathological changes in that organ and there was no dose relationship in the weight changes. No treatment-related changes were seen in absolute or relative weights of the other organs, including testes, ovaries, thyroid, and adrenal glands. There were no treatment-related histopathological findings, and the incidence and grade of microscopic lesions were comparable between treated and control dogs for all organs including testes, ovaries, thyroid, adrenals, and pituitary.

The NOAEL for the study is the high dose of 500 mg/kg/day; a LOAEL was not identified.

Avian Reproduction (OCSPP 850.2300)

In an avian reproduction study (**MRID 00111953**), mature Mallard ducks were administered glyphosate (83% purity) at nominal concentrations of 0, 50, 200, or 1000 ppm (mg/kg) in the diet for 17 weeks (nine weeks prior to the start of egg production and eight weeks of egg production). Each treatment group was comprised of five pens, with five drakes and two hens per pen. Body weights were taken four times: at initiation, after five weeks, prior to onset of egg laying, and at termination of the study. Food consumption was recorded biweekly. Eggs were cleaned at weekly intervals and placed in an incubator. On Days 22 or 23 of incubation, the eggs were placed in a hatcher, and on Days 26 or 27, hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age. For the purposes of measuring egg weight and egg shell thickness, one egg from each pen was randomly selected on a weekly basis.

Ducks treated with glyphosate up to 1000 ppm (limit dose) showed no symptoms of toxicity or behavioral abnormalities for the duration of the study. There was one mortality on study in the high-dose group during Week 12. Because the mortality occurred during the stress of egg production and no gross abnormalities were noted upon gross necropsy, the death was not considered to be treatment-related. There were no treatment-related effects on the reproductive parameters that were evaluated: eggs laid, eggs cracked, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, representative hatchlings body weight, representative 14-day-old survivors body weight, egg weight, and eggshell thickness. The NOAEC is 1000 ppm and the LOAEC is >1000 ppm.

In another avian reproduction study with the mallard duck (**MRID 00036328**), test animals were exposed to concentrations of control, 3, and 30 ppm. There were five pens per treatment group with 2 males and 5 females per pen. Birds receiving glyphosate at 3 and 30 ppm in the diet showed no signs of toxicity or behavioral abnormalities during the course of the study. All test and control birds appeared normal throughout the study and there were no observed mortalities. Additionally, there were no treatment related effects on any reproductive parameter assessed.

In a third mallard duck reproduction study (MRID 48876602), the one-generation reproductive toxicity of Glyphosate-acid to 16 pairs per level of 21-week old Mallards (*Anas platyrhynchos*) was assessed over 21 weeks. Glyphosate-acid was administered to the birds in the diet at nominal concentrations of 0 (negative control), 500, 1000, and 2250 mg ae/kg diet. Mean-measured concentrations were 0 (control), 501, 987, and 2160 mg ae/kg diet. No treatment-related mortalities or signs of toxicity occurred during the test. One incidental mortality was observed in each of the control, 501, and 987 mg ae/kg groups. Significant reductions were detected at the low and high treatment levels for 14-day survivor body weight and adult male weight gain (Dunnett's, $p<0.05$); no effects were detected at the 987 mg ae/kg diet level for any endpoint ($p>0.05$). Reductions in 14-day survivor body weights at the low and high dose were 10-11% of control body weight values and adult male birds did not gain weight at the low dose and lost weight during the study at the high dose. Additionally, the reviewer's analysis detected a suggestively dose-dependent, significant 6% reduction from control in hatchling body weights at the high dose level (William's, $p<0.05$). No other significant treatment-related differences were observed between the control group and any of the treatment groups. Based on the significant reductions from control in offspring survivor body weight and adult male body weight gain at the 501 mg ae/kg diet level, the reviewer concluded that a NOAEL could not be determined in this study (i.e., <501 mg ai/kg diet).

In an avian reproduction study (**MRID 00108207**), mature Bobwhite quail were administered glyphosate (83% purity) at nominal concentrations of 0, 50, 200, or 1000 ppm diet for 17 weeks (nine weeks prior to the start of egg production and eight weeks of egg production). Each treatment group was comprised of 12 pens, with one male and two females per pen. Body weights were recorded at the initiation of the study, after five weeks, immediately prior to the onset of egg laying, and at study termination. Food consumption was recorded biweekly throughout the study. Day 19 eggs were placed in a hatcher. All eggs on day 21 or 22 were removed from the incubator. Hatchlings were housed according to the appropriate parental grouping and maintained on a control diet until 14 days of age. One egg from every other pen was taken weekly for egg weight and eggshell measurements.

Quails treated with glyphosate up to 1000 ppm (limit dose) showed no symptoms of toxicity or behavioral abnormalities for the duration of the study. There were three mortalities during the study in the control group at Weeks 13, 14, and 17. Because the mortalities occurred during the stress of egg production and no gross abnormalities were noted upon gross necropsy, the deaths

were not considered to be treatment-related. There was no effect of treatment on the following reproductive parameters: eggs laid, eggs cracked, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, and representative hatchlings body weight. Decreases in eggshell thickness (50 ppm group; ↓5%, $p<0.05$) and egg weight (1000 ppm group; ↓8%, NS) were not considered to be biologically meaningful, as there was no dose response observed for eggshell thickness decreases and no effect on overall reproductive success. The NOAEC is 1000 ppm and the LOAEC is >1000 ppm.

Fish Early Life-Stage Toxicity (Threespine Stickleback)

In a published non-Guideline study (**Le Mer *et al.*, 2013**), the effects of glyphosate (≥96% purity) exposure on larval threespine sticklebacks (*Gasterosteus aculeatus*) was determined according to growth, survival, the reproductive biomarkers vitellogenin (VTG) and spiggin (SPG), and sexual differentiation as endpoints in exposed larvae. The tested concentrations of 0.1, 1, and 10 µg/L were considered to be representative of environmental concentrations found in estuarine and coastal waters of eastern North America; a higher pharmacological concentration of 100 µg/L also was included.

Adult threespine sticklebacks were collected from an uncontaminated site on the Gaspé Peninsula (Rivière Saint-Jean, QC) and mature adult males and females displaying secondary sexual characteristics were placed separately in 105-L tanks supplied with a constant flow of filtered seawater and maintained at 18±1°C and salinity 15±1%, with a 16:8 hour light:dark photoperiod. After fish were acclimated for ten days, 40 males and females were placed in 45-L tanks containing nesting materials. Mating trials were conducted, and fertilized egg clutches were transferred to 1 L beakers with aerated IML seawater, at 18-19°C, until hatching. Newly hatched larvae, at Day 7 after fertilization, were exposed to the treatments in 1-L glass beakers with 500-mL portions of aerated seawater at 18°C with a 16:8 hour light:dark photoperiod. The tested concentrations were 0.1, 1, 10, and 100 µg/L glyphosate, and the tests included a negative (seawater) control, a positive control of 17α-ethinylestradiol (EE2; 0.05 µg/L), and a positive control of dihydrotestosterone (DHT; 3 µg/L). Each treatment included three replicates, with 9-10 fish per replicate for glyphosate. Fish were exposed throughout the embryo-larvae and juvenile life stages (from hatching to Day 42 post-hatching) until fish had a mean length of ~20 mm. Larvae were fed once per day with *Artemia nauplii*; water was changed every day (>80% replacement) before feeding. Dissolved oxygen, temperature, and fish mortality were monitored daily. Experiments were conducted during the summer of 2007 and repeated during the summer of 2008.

At the end of the 42-day exposure period, surviving fish were sacrificed and total body lengths (L; mm) and wet weights (W; mg) were determined; a condition factor (CF) was calculated as $CF = (W/L^3)100$. Half of the fish from each replicate, selected at random, were frozen at –80°C for VTG and SPG measurements, and the remaining fish were preserved for intersex evaluations;

a subset of these fish were processed for histological evaluation. Vitellogenin was isolated from the whole-body homogenates of juvenile sticklebacks with a previously described method and measured with an indirect competitive ELISA. Stickleback SPG was determined with a competitive ELISA that included an antibody against a synthetic peptide specific to SPG sequence. For histological evaluations, the gonads were examined by light microscopy; all sections were examined for phenotypic sex determination.

In 2007, mean glyphosate concentrations could not be determined due to possible seawater interference. In 2008, mean glyphosate concentrations were 0.08, 1.0, 8, and78 mg/L (≤22% of nominal).

There was <11% mortality in the fish exposed to glyphosate over the 42-day exposure period. There were no significant differences in wet weight, body length, or condition of juvenile fish between glyphosate-exposed fish and control in either test year. The study authors noted that there was a significant difference in the size of fish in the 2007 trials compared with those of the 2008 trials, even though fish came from the same field site; the 2008 fish were smaller (up to 12% less in body length) and weighed less (up to 32% less in wet weight) than the 2007 fish. For the 2007 trial, the condition factor for fish treated with EE2 was similar to all other treatments. In 2008, the condition factor for EE2-treated fish was decreased ($p<0.05$) compared to the DHT treatment and the 1.0 and 100 µg/L glyphosate treatments. Treatment with EE2 resulted in significant ($p<0.05$) induction of whole-body VTG compared to other treatments. There was no VTG induction observed in fish treated with DHT or glyphosate, or in fish in the negative control groups. No induction of SPG was observed in any of the treatment groups, including the positive DHT control.

Glyphosate treatment had no effect on the proportion of mixed sex individuals. A single mixed sex individual was found in the group exposed to 10 µg/L glyphosate. The proportion of mixed sex individuals was higher in the positive controls (EE2, DHT) compared to the negative control. The proportion of female individuals did not differ significantly among treatments, but tended to be higher in the fish exposed to EE2 and the lowest dose of glyphosate, and lower in fish exposed to DHT. For histological evaluations, all sections with gonad tissue were examined for phenotypic sex determination. The number of gonad sections per individual and the proportion of individuals without gonad sections did not differ among treatments. It was concluded that glyphosate exposure had no effect on larval survival or growth, and that glyphosate did not exert estrogenic or androgenic effects on early life-stages of sticklebacks at environmentally-realistic concentrations.

Toxicity to Amphibians (Non-guideline)

In a published non-guideline study (Howe et al, 2004), the effects of glyphosate (reported as technical grade) to leopard frogs (Rana pipeins) was investigated at 0.6 and 1.8 mg glyphosate free acid equivalents (FAE).

The study reports on a series of studies conducted over roughly a seven-year period examining the acute and chronic effects of glyphosate alone, the surfactant polyethoxylated tallowamine (POEA) and glyphosate based products on four aquatic-phase amphibian (anuran) species. It is noted that only the effects of glyphosate alone were considered in the weight of evidence for endocrine related(decreases in offspring body weight observed in one avian reproduction study) effects.

Treatment with glyphosate alone did not reveal any effects on growth (length), tail morphology, percent surviving to reach Stage 42, time to metamorphosis, gonadal development or thyroid hormone gene expression relative to controls; however, treatment with POEA alone or glyphosate formulations containing POEA reduced the percentage of larvae surviving to reach Stage 42, decreased length at metamorphosis, increased time to metamorphosis, and resulted in mixed-sex gonads.

Mortality was relatively high in chronic study control groups (38%) and suggests that environmental conditions were not ideal for promoting survival of leopard hogs. This may have been due to relatively high loading rates coupled with partial water changes every 96 hours. Although effects on metamorphosis and gonadal development appear to be treatment related, poor environmental conditions could affect development.

Fish Life Cycle Toxicity (Fathead Minnows) (OPPTS 850.1500)

In a fish full life-cycle toxicity study (**MRID 00108171**), Fathead minnows (*P. promelas*) were continuously exposed to glyphosate (87.3% purity) at nominal concentrations of 0, 1.6, 3.2, 6.3, 12.5, and 25.0 mg/L; mean-measured concentrations were 0.7, 2.8, 7.0, 13.0, and 25.7 mg/L. In duplicate aquaria for each treatment, two groups of thirty eggs were incubated; dead eggs were removed and counted each day until hatching was completed (4 days at 25°C). Percent hatch was based on the number of live fry from 60 eggs. Forty fish, divided into two groups of twenty each, were randomly selected and distributed to growth chambers in each aquarium. Two growth chambers were used to facilitate handling of fry for 30- and 60-day measurements by a photographic method. Percent survival based on cumulative mortality was also determined at these intervals. After the Day 60 measurements, the number of fish released to each spawning chamber was impartially reduced to fifteen after combining fish from the growth chambers. On Day 64, five spawning sites were placed in the spawning chamber. After secondary sexual characteristics were well developed (approximately Day 134), the number of fish in each tank was reduced initially to four males and four females, and finally to two males and four females on Day 179. Eggs were removed from the spawning sites daily and counted after spawning began (approximately Day 112). Fifty eggs from each of the first ten spawnings per tank were oscillated in their respective test waters by means of an egg cup, and dead eggs were removed and counted each day until hatching was completed (3-5 days at 25°C). Percent hatch was based on the number of live fry from 50 eggs. Twenty fry from the first two spawns in each tank in which at least 80% live hatch was observed were placed in their respective growth chambers. After thirty days of exposure, fry groups were terminated and total lengths determined by the

photographic method. Total wet weight and percent survival were also determined at this time for each fry group.

There was no treatment effect on any of the parameters measured: survival, growth and egg production of first generation fish; and hatchability, survival, and growth of second generation eggs and fry. The NOAEC is 25.7 mg/L and the LOAEC is ≥25.7 mg/L.

APPENDIX 3: References Not Utilized in the Glyphosate WoE Analysis

In 2009, after public review and comment, a final list of 67 chemicals and schedule for issuing Test Orders for the EDSP Tier 1 screening battery was made available in a Federal Register Notice issued October 21, 2009 (74 FR 54422). The agency's review of the initial data submitted as "other scientifically relevant information (OSRI) was provided in the Report of the Endocrine Disruptor Review Team (USEPA, 2010).

Beginning in 2011, the agency has reviewed data cited as "OSRI which included Part 158 studies previously submitted to the agency for registration/reregistration, published literature articles and/or Tier 1 assays. The agency also conducted a more recent search (2009 to 2014) of available scientific literature for any additional relevant information for their weight of evidence (WoE) evaluations. These articles were evaluated in accordance with the agencies Evaluation Guidelines for Ecological Toxicity Data in Open Literature, May 2011 (http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/PDF_rot/esa_evaluation_open_literature.pdf) and the 2012 Guidance for considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (http://www.epa.gov/pesticides/science/lit-studies.pdf).

The following published and unpublished references were considered for use in the WoE analysis for Glyphosate but were not utilized due to one or more of the following reasons: 1) the article was not available in English; 2) the compound of interest was not used in the study; 3) the test material was not adequately described; 4) a formulated end-use product or mixture of chemicals was utilized as the test material; 5) only acute mortality toxicity data were provided; 6) the experimental conditions were not adequately described; 7) only an abstract of the study was available; 8) the reference is a review article or book chapter and does not contain primary study data; 9) insufficient information was available to adequately assess the validity of the study results; 10) the 40 CFR Part 158 guideline study was classified as unacceptable/inadequate; 11) the study dealt only with non-EDSP assay development; 12) no specific endocrine-related endpoints were assessed in the study; and 13) the study contained only data on invertebrates.

Forgacs, A. L., Ding, Q., Jaremba, R. G., Huhtaniemi, I. T., Rahman, N. A., and Zacharewski, T. R. (2012) BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol. Sci.* 127(2):391-402