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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Re: **Endocrine Disruptor Screening Program Tier 1 Screening Order Issuing Announcement (74 FR 54422); EPA–HQ–OPP–2009–0634**

The accompanying comments are being submitted on behalf of the more than two million members and supporters of People for the Ethical Treatment of Animals who are concerned about promoting reliable and relevant toxicity testing strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals. Our comments are submitted in response to issuance of Tier 1 Screening Orders for the Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP) for linuron issued on December 10, 2009, under the request for existing data and "other scientifically relevant information" (OSRI) in which "persons other than recipients" have 90 days to respond.

Introduction

EPA has initiated the EDSP Tier 1 screening for the first group of 67 chemicals by issuing test orders from October 29, 2009, through February 26, 2010. The 67 Phase I chemicals consist of 58 pesticide active and nine High Production Volume (HPV) chemicals used as pesticide inert ingredients (also known as "pesticide inerts"). These chemicals were chosen for testing based on exposure potential considering four exposure pathways for each type of chemical. The four exposure pathways identified for pesticide active ingredients are: food, drinking water, residential use, and occupational exposure. The four exposure pathways identified for HPV/pesticide inert chemicals are: human biological monitoring, ecological biomonitoring, drinking water, and indoor air.[1]

These chemicals are to be tested in five *in vitro* and six *in vivo* assays (**Table 1**). The stated purpose of the Tier 1 battery is to "identify substances that have the potential to interact with the EAT [estrogen/androgen/thyroid] hormonal systems…".[2] The EPA has stated that it intends to use a weight-of-evidence approach to evaluate the results of the Tier 1 studies,[3] and based on this assessment, EPA will determine which, if any, of the Tier 2 tests are necessary. The putative Tier 2 battery consists of developmental and reproductive toxicity tests in several vertebrate species and is designed to identify and establish dose-response relationships for any adverse endocrine-related effects.

These comments address the test orders for glyphosate, a non-selective phosphonomethyl amino acid herbicide that has been extensively tested as part of registration. This testing involves dozens of toxicity tests in vertebrate animals, including reproductive and chronic/lifecycle studies in rodents, fish and birds, as well as metabolism and pharmacokinetics studies.[4] These

tests kill thousands of animals and include many of the same endpoints addressed in the presumptive EDSP Tier 2 tests (**Table 2**).

These comments are a continuation in a series of comments on individual Phase I chemicals from PETA and the Physicians Committee for Responsible Medicine.

References

[1] 74 FR 17579. April 15, 2009; EPA Final List of Initial Pesticide Active Ingredients and Pesticide Inert
Ingredients to be Screened Under the Federal Food, Drug, and Cosmetic Act.

[2] 74 FR54415, October21, 2009. Endocrine Disruptor Screening Program (EDSP); Announcing the
Availability of the Tier 1 Screening Battery and Related Test Guidelines; Notice.

[3] Response to Comments on the Public Review Draft of the Information Collection Request (ECR) entitled "Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP)", contained in Docket ID no. EPA-HQ-OPPT-2007-1081, page 16.

[4] 72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional Chemicals.

Table 1: EDSP Tier 1 Assays

	Species	Mechanism addressed	Endpoints	suggested equivalent information
in vitro				
ER TA: OPPTS 890.1300 OECD TG 455	endogenous human ERα	Estrogen agonists	ERa-dependent transcriptional activation	effect ovary/uterus size, histology, male/female fertility
ER binding OPPTS 890.1250	Rat uterine cytosol	Estrogen agonists, antagonists	ER binding	effect ovary/uterus size, histology, male/female fertility
AR binding: OPPTS 890.1150	rat prostate cytosol	Androgen agonists, antagonists	AR binding	effect on testes size, histology, male/female fertility
Steroidogenesis - H295R OPPTS 890.1550	human	Steroid synthesis (estrogen and testosterone)	testosterone, estrogen hormone levels	effect on estrogen/testosterone levels, sex organs, male/female fertility
Aromatase OPPTS 890.1200	human	Aromatase inhibition, the enzyme responsible for the conversion of androgens to estrogens	$^3H_20$ released during the conversion of androstenedione to estrone	effect on estrogen/testosterone levels, sex organs, male/female fertility
In vivo:				
Uterotrophic OPPTS 890.1600 OECD TG 440	rat, mouse immature: PND 18 - 21 ovarectimized: 6 - 8 weeks	Estrogen agonists, antagonists (in GD, not well developed)	body weight, uterine weight, optional: histolopathology of vagina	evidence of estrogenic activity, uterine or vaginal weight changes, uterine or vaginal histology, effects on fertility reproduction
Hershberger OPPTS 890.1400 OECD TG 441	rat, mouse	Androgen agonists, antagonists, and 5α-reductase inhibitors	ventral prostate (VP), seminal vesicle (SV), levator ani-bulbocavernosus (LABC) muscle, paired Cowper's glands (COW) and the glans penis (GP)	evidence of androgenic activity, male sex organ weights or histology, effects on fertility reproduction

Pubertal female OPPTS 890.1450	rat	Anti-thyroid, estrogenic or anti-estrogenic (including alterations in receptor binding or steroidogenesis), luteinizing hormone, follicle stimulating hormone, prolactin or growth hormone levels or via alterations in hypothalamic function	Growth (daily body weight), Age and body weight at vaginal opening, Organ weights: Uterus, Ovaries, Thyroid, Liver, Kidneys, Pituitary, Adrenals. Histology: Uterus, Ovary, Thyroid, Kidney. Hormones: Serum thyroxine (T4), Serum thyroid stimulating hormone (TSH). Estrous cyclicity: Age at first estrus, length of cycle, percent of animals cycling. Standard blood panel, including creatinine and blood urea nitrogen.	evidence of estrogenic or thyroid activity, uterine or vaginal weight changes, uterine or vaginal histology, effects on fertility reproduction
Pubertal male OPPTS 890.1500	rat	Anti-thyroid, androgenic, or anti-androgenic [androgen receptor (AR) or steroid-enzyme-mediated], alterations in gonadotropins, prolactin, or hypothalamic function	Growth (daily body weight), Age and body weight at preputial separation, Organ weights: Seminal vesicle plus coagulating gs, Ventral prostate, Dorsolateral prostate, Levator ani/bulbocavernosus muscle complex, Epididymides, Testes, Thyroid, Liver, Kidneys, Pituitary, Adrenals. Histology: Epididymis, Testis, Thyroid, Kidney. Hormones: Serum testosterone, Serum thyroxine (T4), Serum thyroid stimulating hormone (TSH). Standard blood panel, including creatinine and blood urea nitrogen.	evidence of androgenic or thyroid activity, male sex organ weights or histology, effects on fertility reproduction
Amphibian metamorphosis OPPTS 890.1100	*Xenopus laevis*	hypothalamic-pituitary-thyroid (HPT) axis, Androgen agonists, antagonists, testosterone synthesis	Day 5: developmental assessment: hind limb and body length, body weight, developmental stage. Day 21 (termination): Developmental stage, SVL, hind limb length and wet body weight, thyroid gland histology.	evidence of androgenic or thyroid activity, male sex organ weights or histology, effects on fertility reproduction
Fish short-term reproductive screen OPPTS 890.1350 OECD 229	fathead minnow	hypothalamus-pituitary-gonadal (HPG) axis	survival, reproductive behavior, secondary sexual characteristics (number and size of nuptial tubercles), gonadal histopathology, gonado-somatic index, plasma concentrations of vitellogenin, 17β-estradiol and testosterone, fecundity (# eggs/female), fertility (%embryos/eggs)	evidence of estrogenic/androgenic activity, effects on fertility of reproduction

Table 2: Pesticide Data requirements related to EDC

Toxicological data requirements			**Use**	
OPPT guideline		**Relevant endpoints**	**food**	**non-food**
870.4100	Chronic oral: rodent	12 months exposure: gross necropsy plus histopathology of liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, thyroid (with parathyroid), spleen, brain	R	CR
870.6200	90-day neurotoxicity	FOB: autonomic function (lacrimation, salvation, etc), convulsions, tremors, abnormal motor movements, reactivity to general stimuli (no reaction to hyperreactivity), general level of activity (unresponsive to hyperactive), posture and gait abnormalities, forelimb and hindlimb grip strength, foot splay, sensorimotor responses, body weight, neuropathology.	R	R
870.4200	Carcinogenicity	24 month exposure: clinical observations, blood smears, gross necropsy, possible histopathology of salivary glands, esophagus, stomach, intestine, liver, pancreas, gallbladder, brain, pituitary, peripheral nerve , spinal cord , eyes, adrenals, parathyroid, thyroid, trachea, lungs, pharynx, larynx, nose. aorta, heart, bone marrow, lymph nodes, spleen, kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries, female mammary gland, all gross lesions and masses, skin.	R	CR
870.3700	Prenatal developmental toxicity, rat and rabbit	Exposure througout gestation: fetal deaths, resoption, sex and weight of each fetus, skeletal and soft-tissue abnormalities of fetuses	R	R
870.3800	Reproduction and fertility	Standard 2-gen: integrity and performance of the male and female reproductive systems, including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, and on the growth and development of the offspring. **P animals:** Cycling in females, sperm count, morphology, motility in males. Organ weights: uterus , ovaries, testes, epididymides, seminal vesicles, prostate, brain, pituitary, liver, kidneys, adrenal glands, spleen. Hisotpathology of vagina, uterus with oviducts, cervix, and ovaries, testis, epididymis, seminal vesicles, prostate, coagulating gland, pituitary and adrenal glands. **F1:** weight and gross abnormalities throughout developement, age of vaginal opening and preputial separation, anogenital distance, same organ weights as P, same histopath as P. **F2 weanlings:** histopathological examination of treatment-related abnormalities.	R	R
870.6300*	Developmental neurotoxicity	Perinatal exposure. Pup weight during growth, gross developmental abnormalities, motor activity, learning and memory, neuropathology (brain)	R	CR
870.7800*	Immunotoxicity	Functional tests: either antibody plaque-forming cell (PFC) assay or ELISA-based antibody reaction, NK cell activity. Cell counts of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations.	R	R

Terrestrial and aquatic non-target organism data requirements			Use				
			terrestrial	aquatic	forestry	residential	Greenhouse/ indoor
850.2300	Avian reproduction	Eggs laid, percent fertilized, eggs not cracked, shell thickness, hatching, chick survival	R	R	R	R	NR
850.1400 (OECD TG 210)	Fish early life stage (freshwater)	Exposure of eggs until hatching: cumulative mortality, numbers of healthy fish at end of test, time to start of hatching and end of hatching, numbers of larvae hatching each day, length and weight of surviving animals, numbers of deformed larvae, numbers of fish exhibiting abnormal behavior.	R	R	R	R	NR
850.1500	Fish life cycle	Locomotion, behavioral, physiological, and pathological effects, spawning, egg numbers, fertility, and fecundity.	CR	CR	CR	CR	NR

*new in 2007

Glyphosate CAS number 1071-83-6

Test order numbers EDSP-417300-227 to 249 and EDSP- 417300-808

Test order date: January 14, 2010; February 25, 2010

Introduction:

Glyphosate is a non-selective phosphonomethyl amino acid herbicide registered for use on many food crops and can also function as a plant growth regulator at low concentrations. It is a contact herbicide and is most often used to control pest species when growing hay, soybeans, and corn. Glyphosate-resistant plants called "Round-up Ready" were introduced several years ago, causing its use to increase dramatically (RED Factsheet 1993).

The nature of glyphosate residue in plants and animals is well understood. In a wide variety of plants studied, glyphosate uptake from soil is limited and the small amount that is taken up is readily translocated throughout the plant. In animals, most glyphosate is eliminated through feces and urine. The primary degradate of glyphosate is amino methyl phosphonic acid (AMPA) and many formulations (like Round-Up®) contain the adjuvant polyethoxylated tallow amine (POEA) (RED Factsheet 1993).

Currently there are three registered iterations of the active ingredient: isopropylamine salt of glyphosate, sodium salt of glyphosate, and monoammonium salt of glyphosate (RED 1993). The active ingredients will be referred to as Glyphosate Active or GA. In addition there are several different formulatory adjuvants. The data presented below includes both GA toxicity testing as well as complete formulations and is specified.

Toxicity and Human Health Concerns:

Glyphosate active (GA), CAS 1071-83-6, is classified in Toxicity Category III and is of relatively low acute toxicity via oral (>4320 mg/kg, rat) and dermal (>2mg/kg, rabbit) exposure (Birch 1970) in rodents and rabbits. In a 2 year feeding study using Sprague-Dawley rats, the NOEL for systemic toxicity is 31.39 (male) and 34.02mg/kg (females) which was the highest dose tested (HDT). ***No effects on vital signs, body weights***, or mortality were noted in the study (Monsanto Co., 1981b). In a 12 month feeding study, Beagle dogs were dosed at 0, 20, 100, or 500mg/kg GA. ***No effects*** were seen at the highest dose tested so the NOEL is 500mg/kg (Reyna 1985). In a 90-day feeding study, CD-1 mice were fed 0, 250, 500, or 2500mg/kg. Reduced weight gain was seen at 500 and 3500mg/kg so the NOEL is 500mg/kg and the LOEL is 3500mg/kg for systemic toxicity (Street et al. 1980). There is no evidence of carcinogenicity or mutagenicity. GA is practically non-toxic to birds (>2000mg/kg, bobwhite quail) and fish (McAllister 1978). However, many end use formulations contain the surfactant polyethoxylated tallow amine (POEA) which is toxic to fish and are labeled and regulated accordingly. Toxicity data for glyphosate active is summarized below in **Table 1** and clearly indicates low toxicity for the active ingredient. There are a few instances where the highest dose tested (HDT) was lower than other experiments but these do not negate the overall trend.

Using internationally accepted methods, principles, and procedures in toxicology, three major health organizations [Health Canada, United States Environmental Protection Agency (U.S. EPA), and World Health Organization (WHO)] have concluded that there are ***no grounds to suggest concern for human health*** (Health and Welfare Canada, 1986, 1992; U.S. EPA, 1993, 1997a, 1998a; WHO, 1994a). Their conclusions are based on the relatively low toxicity of GA as well as the expected exposure amount from the food supply.

Regulatory History:

Glyphosate was first registered in 1974 and EPA issued a Registration Standard for glyphosate in June 1986 (RED Factsheet 1993). Because of its wide use on food crops, EPA conducted a dietary risk assessment for glyphosate active based on a worst case scenario and concluded that chronic dietary risk posed by glyphosate food uses is minimal. A reference dose (RfD) or estimate of daily exposure that would not cause adverse effects throughout a lifetime is 2mg/kg/day (RED 1993).

Mode of Action:

GA inhibits plant growth through interference with the production of essential aromatic amino acids. This pathway for biosynthesis of aromatic amino acids is not shared by members of the animal kingdom; therefore the biological activity of GA is expected to be ***exclusive to plants*** (Williams 2000).

Endocrine:

The data below summarizes existing data to address Endocrine Disruptor Screening Program (EDSP) Tier I endpoints. In a 2000 literature review summary of 180 scientific journals, it was concluded that ***no adverse effects in reproductive tissues*** from animals treated with GA, primary degradate AMPA, or adjuvant POEA in chronic and/or subchronic studies. Results from standard studies with these materials also failed to show any effects indicative of endocrine modulation. The reviewers concluded that the use of Roundup herbicide does not result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals (Williams 2000).

Developmental and Reproductive Toxicology:

A ***reproductive toxicity study*** was conducted with male and female Sprague-Dawley rats which were administered 0, 3, 10 or 30 mg/kg/day of glyphosate continuously in the diet for ***three generations***. The only effect observed was an increased incidence of focal tubular dilation of the kidney (both unilateral and bilateral combined) in the high-dose male F 3b pups. ***No effects on fertility*** were seen. Therefore, the NOEL for systemic and reproductive toxicity is 30 mg/kg/day. The NOEL and LOEL for developmental toxicity are 10 mg/kg/day and 30 mg/kg/day, respectively (Street 1982).

Another ***reproductive toxicology study*** was conducted with Sprague-Dawley rats which were administered 0, 100, 500 or 1500 mg/kg/day of glyphosate continuously in the diet for ***two generations.*** Treatment-related effects ***observed only in the high-dose group*** included: (1) soft stools, very frequent, in the F0 and F1 males and females; (2) decreased food consumption and body weight gain of the F0 and F1 males and females during the growth (premating) period; and (3) decreased body weight gain of the F1a, F2a and F2b male and female pups during the second and third weeks of lactation. Focal tubular dilation of the kidneys, observed in the previous study (Street 1982), was not observed at any dose level in this study. Based on the above findings, the systemic NOEL and LOEL are 10000 ppm (500 mg/kg/day) and 30000 ppm (1500 mg/kg/day), respectively. The reproductive NOEL is 30000 ppm (1500 mg/kg/day; HDT); and the developmental NOEL and LOEL are 10000 ppm (500 mg/kg/day) and 30000 ppm (1500 mg/kg/day), respectively (Reyna 1990).

In another ***developmental toxicology*** study, higher levels of glyphosate active were used. Groups of pregnant Charles River COBS CD rats were dosed with glyphosate active orally by gavage as a single daily dose on days 6 through 19 of gestation and in subsequent generations. Reduced mean maternal body weight gain was noted in the ***3500 mg/kg/day*** dose group over the treatment period due to mean maternal body weight loss during the first 3 days of treatment. At 3500 mg/kg/day a statistically significant increase in the mean number of early resorptions resulted in a slight increase in mean postimplantation loss. A statistically significant decrease in the mean number of total implantations, viable fetuses, and mean fetal body weight and a slight decrease in the mean number of corpora lutea was noted in this group. Based on these findings***, the NOEL and LEL for maternal toxicity are 1000 and 3500 mg/kg/day, respectively***. An increase in the number of litters and fetuses with unossified sternebrae was noted in the 3500 mg/kg/day dose group. Based on this finding, the NOEL and LEL for developmental toxicity are 1000 and 3500 mg/kg/day, respectively (Monsanto Co., 1980a MRID 00046362 and Tasker, 1980a)

Glyphosate was tested for ***developmental toxicity*** in rabbits following administration by oral gavage at dosage levels of 0, 75, 175, or 350 mg/kg body wt/day from gestational days 6 through 27. Frequent diarrhea was noted in several high-dose animals. Although there were ***no effects in fetuses at any dosage level***, the NOAEL for developmental toxicity was considered to be 175 mg/kg body wt/day due to the insufficient number of litters available for examination in the 350 mg/kg body wt/day dosage group (MRID 00046363 and Tasker, 1980b). The 175 mg/kg body wt/day dosage level was also concluded to be the NOAEL by the WHO (1994a), while the U.S. EPA (1993) considers this level to be the NOEL.

Reproductive and developmental data over a wide range of doses show that GA ***does not cause fertility or reproductive effects*** in rodents or rabbits at doses relevant to humans.

In vitro assessment of steriodogenesis, aromatase activity, androgen and R binding and estrogen receptor-mediated transcriptional activity:

In an *in vitro* assay, researchers used mouse MA-10 Leydig tumor cells to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. Roundup significantly ($p < 0.001$) disrupted steroidogenesis over time without inducing a parallel decrease in total protein synthesis. Interestingly, the active ingredient in Roundup®, GA, ***did not alter steroidogenesis*** or total protein

synthesis at any dose tested (0-100 µg/mL). Researchers indicated that Roundup® decreased steroidogenesis by disrupting StAR expression post-transcriptionally (Walsh 2000).

Using a human hepatic cell line (HepG2), researchers measured cytotoxicity using three aromatase assays (Alamar Blue®, MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic and anti-androgenic effects using gene reporter tests. Liver cells are sensitive to toxins as they function in detoxification *in vivo*. Results confirmed that the nature of the adjuvant in commercially available formulations has a greater affect on toxicity than the amount of glyphosate active. ***Aromatase activity was not affected*** by GA alone. The *in vitro* responses were dependent on formulation variations and not dose dependent with regard to GA concentrations. GA alone had ***no anti-estrogenic activity*** but did have a ***slight anti-androgenic effect*** *in vitro* (Gasnier 2009).

In another *in vitro* study, glyphosate active and Roundup® were applied to human placental JEG3 cells to determine endocrine activity, specifically on aromatase. Cytotoxicity increased with time (8-fold at 0.8% between 24 and 48 hr), and the median lethal dose (LD50) was approximately 1.8 times lower for Roundup® (0.7%) than for glyphosate active. After 1 hr of incubation, estrogen synthesis was enhanced by about 40% with Roundup but not with glyphosate active. After 18 hr of incubation, aromatase activity *in vitro* was inhibited, again with Roundup® only. This inhibition of aromatase activity is assumed to be an effect on aromatase gene expression, attributed to the adjuvant and not GA (Richard 2005).

In another study, glyphosate active was tested in two complementary assays: one measuring activation of the estrogen receptor from rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had ***no estrogenic activity*** in either assay (Petit *et al.* (1997).

The data indicate **no effect *in vitro* on aromatase and estrogen *with perhaps a slight anti-androgenic effect for*** GA. Adjuvants used in commercial formulations, however, may affect endocrine activity.

Estrogenic activity *in vivo*:

In a female pubertal study, an increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (3393mg/kg/day or 50,000 ppm) (NTP 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.* (1988) to conclude that "stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity." Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high doses, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage (NTP, 1992). It is also important to note that these dose levels are several orders of magnitude greater than any exposure ever likely to be experienced by humans.

Androgenic and testosterone activity *in vivo*:

In a subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations (20% below control) were reported in F344 rats at both the 1638 mg/kg (25,000 ppm) and the 3393 mg/kg

(50,000 ppm) levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al.*, 1988). As the apparent reductions were not related to dosage or accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, ***male fertility was not reduced*** in the reproduction study even at the highest dietary level tested (30,000 ppm) (NTP 1992).

In a ***pubertal*** study, male weanling Wistar rats were dosed at 5, 50, or 250mg/kg of Roundup®. Body weight was not affected although a significant delay in puberty, significant weight decrease of adrenals, and a slight decrease in testosterone levels was seen in all three treatment groups. No pathological altercations of adrenals were seen and corticosterone and estradiol levels were not different. Researchers concluded that the direct action of the active ingredient and was not likely the major cause of the puberty delay and that glyphosate active did not present harmful effects on fertility, but instead showed effects for its adjuvant components. (Romano 2010).

The data indicate that GA does not affect male fertility or hormone levels.

Amphibians and Fish:

Tables 2 and 3 summarize available amphibian and fish data. GA is much less toxic than Roundup® to both fish and amphibians, and there is ***no evidence of endocrine activity***.

When comparing glyphosate active with a commercially available formula (e.g. Round-Up), researchers show that there is a significant difference in toxicity. When exposed to GA, *Oncorhynchus mykiss* (rainbow trout) had a 96-h LC50 range of 148-211 mg/L whereas the Roundup formulation was 14 mg/L (Takacs 2002).

Three tests on fish species, one bluegill and two with fathead minnow, showed LC50s of 120 ppm, 84.9 ppm, and 97 ppm, respectively. Two rainbow trout 96-hour LC50 tests provided values of 86 ppm and 140 ppm. Based on these tests, glyphosate active ranges from slightly to practically non-toxic to freshwater fish species (McAllister and Forbis 1978, ID #234395; EG & G Bionomics 1975, ID #00108171 and Folmar, Sanders, and Julin 1979, ID #249160).

Juvenile Rainbow trout (sex not specified) were exposed to 0.11 mg/L glyphosate active. Glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish (Xie 2005).

Birds:

In birds, the toxicity of GA is similar to that seen in other species. In subacute dietary toxicity experiments using bobwhite quail and mallard ducks, reproductive impairment (unspecified) was seen at levels above 4640 ppm (Study IDs 94171 and Fink 1973a).

In another experiment, finches eating seed treated with 2.5 g/kg glyphosate survived the 5-d study period without a reduction in food intake or any overt ill effects. The acute toxicity is equally low in other

species of birds (Japanese quail, Northern bobwhite and mallards) with an LC50 of > 4.6 g/kg (Takacs 2002).

Other Species:

In addition to the species listed above, other mammals have been exposed to Roundup® via oral dosing. The marsupial *Sminthopsis macroura* (Gould), and two species of hopping-mouse, *Notomys alexis* Thomas and *Notomys mitchelli* were fed a diet in which the concentration of glyphosate was increased from 625 μg/g to 5,000 μg/g by doubling the concentration of glyphosate in the food every few days during a 23-d period. The only toxic effect observed in the mammals was a marked body weight loss in the treated *N. alexis.* The data indicate that glyphosate for these four species is probably not or only slightly toxic (Evans 1986).

Summary and Recommendations:

After reviewing the existing data for both GA and commercially available formulations, there ***is no evidence that the active ingredient causes endocrine disruption***. The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are ***not reproductive toxicants*** and ***do not perturb the endocrine system***.

The U.S. EPA (1998a) reviewed these studies and also concluded that there was ***no evidence to suggest that glyphosate produces endocrine-modulating effects***. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. ***No additional data is needed*** to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects.

Table 1: Glyphosate Active NOAELs for Toxicological Endpoints (Williams 2000).

Type of Study	Species Tested	NOAEL (mg/kg/day)	Observations	Study Reference
Subchronic toxicity 90-day	mouse	2310	Decreased weight gain	Tierney, 1979
Subchronic toxicity 90-day	mouse	630	Salivary gland lesions	NTP, 1992
Subchronic toxicity 90-day	rat	1445	No adverse effects at HDT	Stout, 1987
Subchronic toxicity 90-day	rat	209	Salivary gland changes at the lowest dose tested not significant	NTP, 1992
Subchronic toxicity 12-months	dog	500	No adverse effects at HDT	Reyna and Ruecker, 1985
Chronic toxicity 24-month	mouse	885	Liver effects	Knezevich, 1983
Chronic toxicity 26-month	rat	33	No adverse effect at HDT	Lankas, 1981
Chronic toxicity 24-month	rat	409	Decreased eight gain and ocular lesion	Stout and Ruecker, 1990
Developmental Toxicity	rat	1000	Maternal and fetal effects	Tasker, 1980a
Developmental Toxicity	rabbit	175	Maternal toxicity	Tasker, 1980b
Reproductive Toxicity	rat	30	No adverse effects at HDT	Schroeder, 1981
Reproductive Toxicity	rat	694	Systemic toxicity; no reproductive effect	Reyna, 1990

Table 2: LC50 (mg/L) of Glyphosate Active (GA) and Roundup® on several species of tadpoles (Mann 1999)

Organism	Scientific Name	Glyphosate (48-h)	Roundup ® (48-h)
Pobblebonk Frog tadpole	*Lymnodynastes dorsalis*	>400	3
Western Green Tree Frog tadpole	*Litoria moorei*	>343	2.9
Moaning Frog tadpole	*Heleioporus eyrei*	>373	6.3
Brown Froglet tadpole	*Crinia insignifera*	>466	3.6

Table 3: Comparison of the acute toxicity of glyphosate active, Roundup formulation, and the surfactant used in Roundup (Trotter 1990).

Organism	Scientific Name	LC50 (mg/L) 96-h Glyphosate	LC50 (mg/L) 96-h Roundup	LC50 (mg/L) 96-h Surfactant
Rainbow trout	*Oncorhynchus mykiss*	86	8.3	2.0
Fathead minnow	*Pimephales promelas*	97	9.4	1.0
Channel catfish	*Ictalurus punctatus*	130	16	13
Bluegill sunfish	*Lepomis macrochirus*	120	5.0	2.0

References:

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE. (2007). Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contaminants and Toxicology 53(1):126-33.

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