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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 and the Physicians Committee for Responsible Medicine 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 permethrin issued on November 19, 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 permethrin,[4,5] a widely used pyrethroid pesticide, 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.[6] These tests kill thousands of animals and include many of the same endpoints addressed in the presumptive EDSP Tier 2 tests (**Table 2**).

In its letter to EPA approving the Information Collection Request, OMB instructed EPA to "promote and encourage test order recipients to submit Other Scientifically Relevant Information (OSRI) *in lieu* of performing all or some of the Tier I assays, and EPA should accept OSRI as sufficient to satisfy the test orders to the greatest extent possible." In the interest of increasing the efficiency of the EDSP, the comments before provide existing data and OSRI in support of these OMB instructions to EPA, focusing on animal testing and vertebrate testing in particular. There is one section for each of the seven chemicals; references follow each section.

In all cases, the equivalent of Tier 2 (reproductive toxicity in one or more generations) information is available for rodents and in some cases also for fish and birds. There are two primary reasons for carrying out Tier 1 testing: 1) to discern mechanistic information about a chemical (i.e. does it function by interacting with the E, A or T hormone system) and 2) to evaluate what, if any, Tier 2 testing is warranted. Thus, if Tier 2 data already exist for a chemical, there is very little rationale for performing Tier 1 testing.

EPA has not articulated how endocrine disrupting chemicals would be regulated based on mechanism of action. Even though there is no precedence for such regulation to date, future regulation may benefit from mechanism of action information; in fact such information is critical for reduced dependency on whole animal testing and for improving the accuracy of hazard and risk determination as outlined in the 2007 NRC report: Toxicity Testing for the 21st Century: a Vision and a Strategy.[7] Rather than using whole animal tests such as the uterotrophic or Hershberger simply because they are available, mechanistic information can be obtained through non-animal means, in binding, transcriptional activation, or other cell-based systems, some of which are in use by the EPA's ToxCast™ program. A more efficient structure for the EDSP would be to start with a series of mechanistic *in vitro* assays to determine which, if any, of the endocrine pathways a chemical interacts with, and target any further testing accordingly.

The EPA's ToxCast™ program profiled 56 of the 73 EDSP Phase I chemicals in 14 assays directly related to endocrine activity (including estrogen, androgen, thyroid, and aromatase), and in an expanded set of 78 high throughput assays, including nuclear receptor and CYP450-related assays.[8] The advantage of the structure of the ToxCast™ program's database is that connections can rapidly be made between *in vitro* assay results and existing mammalian and ecotoxicity data, which greatly facilitates identification and interpretation of mechanism of action information.

Preliminary results from Phase I of the entire ToxCast™ program are promising.[9] Linkages between high-throughput *in vitro* results and *in vivo* endpoints can be made, and potency rankings for groups or classes of chemicals are also being explored. Intriguingly, high "activity" across a large number of molecular pathways correlates inversely with lowest observed effect level (LOEL) in mammalian studies.

Rather than a default application of the full battery of Tier 1 assays to data-rich chemicals such as pesticides, a more efficient and potentially more useful approach would be to evaluate the existing relevant data, reproductive and developmental information in particular, in combination with information from a series of *in vitro* mechanistic assays such as those included in the Tier 1 and in ToxCast™, to determine what, if any, further testing is warranted.

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] 74 FR 54422, October 21, 2009; Endocrine Disruptor Screening Program; Tier 1 Screening Order Issuing Announcement, Order Issuance Schedule.

[5] EPA Endocrine Disruptor Screening Program, status of EDSP Orders/DCIs (http://www.epa.gov/endo/pubs/edsp_orders_status_012810.pdf; accessed 3 February 2010)

[6] 72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional Chemicals.

[7] NRC (Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies Press, Washington, DC. Available at: http://www.nap.edu/catalog.php?record_id=11970. Accessed 25 January 2009.

[8] Kavlock et al. (2009) Biological Profiling of Endocrine Related Effects of Chemicals in ToxCast™. Poster presentation available at http://www.epa.gov/NCCT/toxcast/files/summit/40P%20Kavlock%20TDAS.pdf. Accessed February 4, 2010.

[9] Judson et al. (2009) "The Toxicity Data Landscape for Environmental Chemicals" Environmental Health Perspectives, Volume 117, Number 5, May 2009 (http://ehp.niehs.nih.gov/members/2008/0800168/0800168.pdf, accessed 5 February 2010).

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

Permethrin, CAS number 52645-53-1

Test order numbers EDSP-109701-83 through 92

Test order date: November 19, 2009

I. Introduction

Permethrin, a broad-spectrum synthetic pyrethroid pesticide, was initially registered in 1979 for use on numerous food and feed crops, livestock and livestock housing, mosquito abatement programs, indoor and outdoor residential spaces, pets, and clothing. Pyrethroids as a class are generally understood to share a common primary mechanism of toxicity by modifying the normal biochemistry and physiology of nerve membrane sodium channels, although subgroups may share slightly variable patterns of neurobehavioral affects (EPA 2009; FIFRA SAP 2009).

Pyrethroids have been experiencing rising popularity, likely as a result of several favorable attributes. While generally perceived as exhibiting low toxicity among non-target species (Soderlund D. et al. 2002; Wolansky M. and Harrill J. 2008), low-volatility pyrethroid pesticides also have a tendency to bind to soil particles where they are degraded within days to months, and as a result are rarely detected in ground waters (USGS 2006). This class of pesticides demonstrates low acute toxicity in birds and mammals, but permethrin itself, while only soluble in water at less than one part per million, is highly acutely toxic to fish and aquatic invertebrates (EPA 2002; EPA 2009).

Most human exposures to permethrin are expected to occur from consumption of treated food products or from domestic product applications. While estimated intakes from diet and drinking water are below recommended limits, occupational exposure is possible via dermal and inhalation routes during mixing and spraying. While pyrethroids are not well absorbed through the skin (ATSDR, 2003; Woollen B. et al. 1992), they are rapidly metabolized following dermal or inhalational absorption and gradually eliminated over several days in urine and bile (Leng G. et al. 1997; Soderlund D. et al. 2002; Woollen B. et al. 1992). Unmetabolized pyrethroids have been measured in breast milk, but may be only poorly transferred across the placenta (ATSDR, 2003; CDC, 2009).

Permethrin has also been approved as a miticide for direct application to the human body, as a 1% solution, since 1986. Clinical trials show that only 0.30 to 2.08 percent of the applied permethrin dose is absorbed through the skin, and that an excess of 80 percent of the applied dose can be removed by washing exposed body surfaces (Meinkin T. et al. 1996; Franz T. et al. 1996). Exposure of newborn mice to permethrin indicates, however, that exposure during the period of lactation may affect the development of the cerebellum (Imamura L. et al. 2002). Although there are no studies to date on the safety of long-term

permethrin exposure during human pregnancy, the chemical's low systemic absorption suggests a low risk of possible developmental impacts as corroborated by epidemiological studies of expectant mothers exposed to permethrin via short term use of topical products (Kennedy D. et al. 2005). Studies of human volunteers have found that a cumulative maximum 14-day dose following use of a 1% shampoo solution at approximately 5.5 mg (WHO 1990).

Consumption of permethrin residues in food products is the most likely route of exposure for most populations. Using the Total Diet Study within the FDA's chemical contaminant monitoring program, the average daily intake of permethrin suggested a range of permethrin exposure between 5.5 and 44.1 ng/kg bodyweight/day, depending on age. The youngest participants in this study, infants between six and eleven months old, experienced the highest rates of exposure (Gunderson E. 1995). Examination of 5,728 groundwater samples within the National Drinking Water Contaminant Occurrence Database detected permethrin in only three samples at an average concentration of 0.011 µg/L, with a maximum concentration of 0.02 µg/L (EPA 2000). Additional epidemiological findings that the developing fetus and children are exposed to measurable levels of pyrethroid pesticides raises concern over the potential for developmental neurotoxic effects (Sheets L. 2000; Morgan M. et al. 2007; Lu C. et al. 2006; Lu C. et al. 2009). ***EPA cites a developmental neurotoxicity study using rats as a source for an acute neurotoxic LOAEL at 75 mg/kg/day, based on clinical signs of aggression and otherwise abnormal behavior (EPA 2009). A 96-day study using dogs established a NOAEL at 50 mg/kg/day based primarily on liver and neurological signs (FAO/WHO 1999).***

Acute mammalian neurotoxicity of pyrethroids has been well characterized, (DeRay 2001; Kaneko H. and Miyamoto J. 2001; Narahashi T. 2001; Shafer T. et al. 2005; Soderland D. et al. 2002). Data pertaining specifically to permethrin suggests that at least some of the developmental, neurotoxic and behavioral effects following exposure may be indirectly due to maternal weight loss during development rather than as a result of direct compound effects. Twenty day exposure of F0 mice of both sexes suggests a developmental NOEL of 4.9 mg/kg/day. However, because permethrin is so rapidly metabolized and excreted, authors note that "these effects cannot be attributed directly to the developmental neurotoxicity of permethrin on F1-pups." Maternal toxicity and subsequent decreases in maternal weights may have been the trigger for the decreased pup weight gain in the middle and high dose groups (3.8 and 7.9 mg/kg/d, respectively). Neuromuscular parameters evaluated in the offspring could be altered, then, as a secondary response to such pronounced stunted growth (Farag A. et al. 2006). The 4.9 mg/kg/day NOEL dose is about 98 times the 0.05 mg/kg/day acceptable daily intake for humans for the *cis*-:*trans*-permethrin mixture (FAO/WHO 1999).

A. Reregistration Eligibility Decision, 2009

The toxicity database for permethrin is extensive and includes several mammalian multigenerational studies (see Appendix A for a full list of toxicity studies examined for permethrin's 2009 RED). ***In***

addition, EPA has stated high confidence in a NOEL and LOEL of 5 mg/kg/day (100 ppm) and 25 mg/kg/day (500 ppm), respectively. This LOEL value was established from a two-year feeding study using rats in which the critical observed effects were increased liver weights (EPA 1992). The EPA has acknowledged the concern for potential developmental neurotoxicity based on this evidence in conjunction with a subchronic neurotoxicity study in rats.

While requiring a confirmatory developmental neurotoxicity (DNT) study in the 2009 RED, the EPA notes, however, that "a dose-analysis with the existing reliable toxicity data for permethrin, that included an evaluation of the acute and subchronic neurotoxicity studies in addition to the three-generation reproduction study, indicates that the results of the DNT would not have an impact on the risk assessment." ***Developmental and reproductive toxicity studies show that there is no qualitative or quantitative evidence for increased susceptibility to infants and children following in utero and or perinatal exposure to permethrin. Additionally, "[i]n the available toxicity studies on permethrin, there was no toxicologically significant evidence of endocrine disruptor effects" (EPA 2009).***

Furthermore, EPA has classified permethrin as a likely human carcinogen based on reproducible lung and liver tumors in experiments using mice along with supporting structural activity relationships (SAR) information. Equivocal evidence for carcinogenicity in Long-Evans rats did not contribute to this classification (EPA 2009).

II. Existing toxicological and experimental data related to endocrine function

Results from studies exploring potential endocrine impact of permethrin exposure are conflicting and vary according to test system, including the choice of animals used in experiments and the treatment routes selected (Tyler C. et al. 1998; Garey J. and Wolff M. 1998; Saito K. et al. 2000; Kunimatsu T. et al. 2002; Chen H. et al. 2004). Overall, available data suggest that permethrin can exert estrogen-like effects in female rats and antiandrogen-like effects in male rats. Several *in vitro* studies suggest presence of permethrin's estrogenic effects in some human cell lines, including MCF-7 and in a human estrogen receptor yeast screen assay (Go V. et al. 1999; Tyler C. et al. 1998). However, other experiments have shown no compound effect in human breast cancer cell lines or in a yeast two-hybrid assay (Nishihara et al. 2000; Saito K. et al. 2000). Results from these studies suggest that permethrin's estrogenic effects may be mediated in part via the c-Neu pathway. Kim et al., using a protocol similar to the current OECD Test Guideline Hershberger protocol have noted that repetition of the Hershberger and Uterotrophic assays with slight variations in protocol is capable of demonstrating potential androgenic activity where previous assays had suggested a lack of effect. Since the Uterotrophic and Hershberger assays are both planned as part of EDSP, conducting further tests would duplicate existing data with little confidence in measurement of a relevant biological effect (Kim S. et al. 2005).

A. Assessment of estrogenic activity

Results from recent studies assessing estrogen receptor (ER)-associated mechanisms using *in vitro* and *in vivo* short-term assays suggest that permethrin is capable of inducing estrogenic effects via the ER. In a three-day uterotrophic assay using ovariectomized rats, permethrin doses between 37.5 and 150 mg/kg/day identified no significant changes in uterine weights. This was the first known *in vivo* study, and previous *in vitro* studies showed inconsistent results (Kunimatsu T. et al. 2002). ***A similar Uterotrophic assay covering a much broader dose range, however, found a statistically significant increase in uterine weights at and above 200 mg/kg/day, and the permethrin-induced change in weight was inhibited by coadministration of an antiestrogen. (Kim S. et al. 2005)*** A concurrent uterine gene expression assay following permethrin exposure identified induction of CalbindinD_{9k} (CaBP-9k), an estrogen-responsive uterine intracellular calcium-binding protein, both with and without coadministration of estradiol (Kim S. et al. 2005). While results are inconsistent, permethrin is clearly capable of interacting with ER-mediated processes under certain conditions at very high dose ranges.

B. Assessment of androgenic activity

Androgen receptor (AR)-associated mechanisms have also been examined using *in vitro* and *in vivo* short-term assays, and recent studies are also suggest a capacity for permethrin to impact growth in androgen-sensitive tissue. In a 5-day castrated Hershberger assay, permethrin administered at doses between 20 and 75 mg/kg/day showed no androgenic or antiandrogenic effects. These results were unchanged when permethrin was coadministered with testosterone propionate. The 75 mg/kg/d dose was the highest anticipated daily dose that could be administered without causing "excessive system toxicity." This was the first known *in vivo* study, and *in vitro* studies up to 2002 showed inconsistent results (Kunimatsu T. et al. 2002). ***A later similar Hershberger assay, however, found a statistically significant decrease in the weights of androgen-dependent sex accessory tissues at all doses tested, from 10 to 100 mg/kg/day, suggesting permethrin's capacity to exert antiandrogenic effects (Kim S. et al. 2005).*** In a separate six week study using adult male ICR mice administered up to 70 mg/kg/d *cis*-permethrin, caudal epididymal sperm count and sperm motility in treated groups were statistically reduced in a dose-dependent manner. Testicular testosterone production and plasma testosterone concentration were significantly and dose-dependently decreased with an increase in luteinizing hormone, and a significant relationship was observed between testosterone levels and *cis*-permethrin residues in individual mice testes after exposure; however, no significant changes were observed in body weight, reproductive organ weights, sperm morphology, or plasma follicle-stimulating hormone concentration. *cis*-Permethrin exposure also significantly reduced testicular mitochondrial mRNA expression of peripheral benzodiazepine receptor (PBR), steroidogenic acute regulatory protein (StAR), and cytochrome P450 side-chain cleavage. Mitochondrial membranes and Leydig cells also exhibited damage in permethrin-exposed mice, suggesting a possible mechanism for the disruption of testosterone biosynthesis. By diminishing the transport of cholesterol into mitochondria and subsequently decreasing its conversion to pregnenolone, testosterone production is likely diminished following permethrin-mediated damage (Zhang S. et al. 2007). Permethrin's *trans* isomer had no significant adverse effects on the mouse reproductive system. (Zhang S. et al. 2008). Commercial formulations of a *cis*-:*trans*-permethrin mixture, however, are capable of interfering with AR-mediated processes at high dose ranges.

C. Assessment of thyroid activity

Direct examination of permethrin's capacity to impact thyroid hormone systems is not extensive, but evidence does imply thyroid dysregulation following exposure. Serum and brain tissue examinations using rats administered 100 to 400 mg/kg/day permethrin have suggested that high dose exposure alters thyroid hormone profiles. Permethrin induced a dose-dependent decrease in the serum levels of T4, T3 and an increase in the serum TSH levels. Permethrin exposure also reduced levels of T4 and T3 in homogenates of the cerebral cortex and hippocampus, respectively, suggesting that permethrin-induced neurotoxicity may involve, in part, an impairment of the physiological action of thyroid hormones on their subcellular targets (Wang S et al. 2002). While permethrin seems to be capable of interfering with thyroid hormone ratios, these effects seem to occur only at doses far greater than is considered relevant to human or wildlife exposures.

D. Amphibians, fish, birds and other ecotoxicological data

There is little information directly examining the role of permethrin-mediated toxicity in birds, probably as a result of the perceived low toxicity of pyrethroids in avian species (EPA 2002; EPA 2009). Aquatic organisms, however, are drastically more susceptible to permethrin's toxic and developmental effects (EPA 2002; EPA 2009).

Some aquatic species demonstrate developmental impacts at relatively low concentrations. Larval shrimp were the most sensitive life stage with a 96-h lethal concentration (LC50) of 0.05 mg/L, compared to 0.25 mg/L for adults, and 6.4 mg/L for embryos. The presence of sediment, to which permethrin binds, significantly decreased the toxicity of permethrin to both adult and larval shrimp. Permethrin exposure increased time to hatch in embryos and decreased swimming behavior of larvae (DeLorenzo M. et al. 2006). These results indicate that very low levels of the synthetic pyrethroid permethrin may negatively affect grass shrimp health and survival. Permethrin was found to have an aqueous half-life ranging from 2.5 to 4.6 days, which is within the exposure period used in the grass shrimp toxicity tests (Schimmel S. et al. 1983). The expected environmental concentration for permethrin is approximately 0.06 mg/L for mosquito control applications and ranges from 0.51 to 0.95 mg/L for agricultural crop applications (EPA 2005; DeLorenzo M. et al. 2006), which are above limits known to cause adverse developmental effects in shrimp.

Developmental impacts have also been noted in fish. Exposure of zebrafish embryos at doses approaching the LC50, permethrin causes craniofacial abnormalities at 200 µg/L and above (DeMicco A. et al. 2010). These findings are consistent with mammalian studies demonstrating that pyrethroids are mildly teratogenic at very high doses. However, at lower doses, body axis curvature and spasms were observed, which were reminiscent of the classic neurological syndromes observed with pyrethroid toxicity. ***Treatment***

with the sodium channel antagonist MS-222 ameliorated both spasms and body curvature, suggesting that pyrethroid-induced neurotoxicity is similar in zebrafish and mammals (DeMicco A. et al. 2010). Furthermore, species-specific variations in sodium channel bioforms would be expected to modulate potential developmental toxicities among animals used in testing and, indeed, humans exposed to environmental concentrations of permethrin (Meacham C. et al. 2008; Soderlund D. et al. 2002).

III. Summary and recommendations

The existing permethrin database contains ample data indicating an array of neurotoxic and developmental effects. Direct and indirect endocrine effects have been defined in both sexes among several species. Permethrin has been thoroughly tested in a wide range of vertebrate species using diverse methods, including protocols identical or similar to those required under Tier 1 of the EDSP as well as several tests similar to those proposed for Tier 2, including the rodent two generation reproductive toxicity test as part of registration and reregistration. Neurotoxic NOAELs have been described in tests on mammalian species in spite of a lack of consistent mechanism of action findings, as have NOELs for other endocrine-related endpoints. While additional testing using existing *in vitro* methods may provide a renewed opportunity to investigate possible mechanisms underlying permethrin's putative endocrine effects, the downstream biological effects of any endocrine disrupting mechanism are nevertheless associated with dose ranges that are not relevant to humans. However, there is sufficient evidence that permethrin exposure can and does occur at levels that cause adverse ecological effects; exposure should be regulated based on this existing information without requiring additional testing. There is therefore no justification for further *in vivo* testing of permethrin as part of the EDSP.

IV. References

1. Centers for Disease Control and Prevention. 2009. *"*Fourth National Report on Human Exposure to Environmental Chemicals*."* http://www.cdc.gov/exposurereport/. (Accessed February 17, 2010).
2. Chen H. et al. 2004. "Estrogenicity of organophosphorus and pyrethroid pesticides." J. Toxicol. Environ. Health. 65: 1419-1435.
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Appendix A: Guideline tests performed as part of parathion's 2009 revised RED

OPPTS Guideline	Study Title
Exotoxicity Data	
850.2300 (A & B)	Avian Reproduction
850.1075 (A & B)	Fish Acute Toxicity - Freshwater
850.1010 (A,B,C & D)	Acute Aquatic Invertebrate Toxicity
850.1400	Early Life-Stage Fish (Freshwater)
	Early Life-Stage Fish (Marine)
850.1300 (72-4B)	Life-Cycle Aquatic Invertebrate
Mammalian Toxicology	

870.1100	Acute Oral - Rat
870.1200	Acute Dermal - Rabbit
870.1300	Acute inhalation toxicity
870.2400	Primary Eye Irritation - Rabbit
870.2500	Primary Dermal Irritation - Rabbit
870.2600	Dermal Sensitization
870.3100	90-day feeding - Rodent
870.3150	90-day feeding – Non-rodent (Dog)
870.3200	21-Day Dermal Toxicity - Rat
870.3700	Developmental Toxicity - Rat
870.3700	Developmental Toxicity - Rabbit
870.3800	Reproduction and Fertility Effects - 2 Generation Repro
870.4300	Chronic Feeding Toxicity - Rodent Combined Chronic Toxicity/Carcinogenicity
870.4200	Oncogenicity - Rat
870.4200	Oncogenicity - Mouse
870.5100	Bacterial Reverse Mutation Assay
870.5395	In vivo mammalian cytogenetics tests: erythrocyte micronucleus assay
870.5550	Other Genotoxic Effects
870.7485	General Metabolism - Rat
870.7485	General Metabolism - Dog
870.6200	Subchronic neurotoxicity screening battery
870.6300	Developmental Neurotoxicity Study
870.7600	Dermal Absorption in rats

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