



PEOPLE FOR THE ETHICAL
TREATMENT OF *ANIMALS*

HEADQUARTERS

501 FRONT ST.
NORFOLK, VA 23510
757.622.PETA | 757.628.0781 (FAX)
PETA.org | StopAnimalTests.com



5100 WISCONSIN AVENUE, N.W., SUITE 400
WASHINGTON, DC 20016
T: (202) 686-2210 F: (202) 686-2216
PCRM@PCRM.ORG WWW.PCRM.ORG

Office of Pesticide Programs
US Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington, DC 20460-0001

6 February 2010

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 the first seven chemicals, issued on October 29, 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 the first seven chemicals: atrazine, 2, 4-D, benfluralin, dimethyl tetrachloroterephthalate (DCPA), fenbutatin oxide, norflurazon, and

propargite.^{4,5} All seven of these chemicals are herbicide or insecticide active ingredients, and are therefore subject to extensive testing for pesticide 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 ToxCastTM 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 ToxCastTM program profiled 56 of the 73 EDSP Phase I chemicals, including atrazine, 2,4-D, benfluralin, norflurazon and propargite, 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 ToxCastTM 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 ToxCastTM 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, October 21, 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
<i>in vitro</i>				
ER TA: OPPTS 890.1300 OECD TG 455	endogenous human ER α	Estrogen agonists	ER α -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	³ H ₂ O released during the conversion of androstenedione to estrone	effect on estrogen/testosterone levels, sex organs, male/female fertility
<i>In vivo:</i>				
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: histopathology 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 5 α -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	<i>Xenopus laevis</i>	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, gonadosomatic index, plasma concentrations of vitellogenin, 17 β -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, salivation, 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 throughout gestation: fetal deaths, resorption, 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. Histopathology 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 development, 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 e/ 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

Atrazine, CAS number 1912-24-9
Test order numbers EDSP-080803-1 through 4
Test order date: October 29, 2009

I. Introduction

Atrazine, a chlorotriazine herbicide, has been on the market for over 50 years (first registered in 1958) and is one of the most widely used herbicides in the US and one of the most widely used agricultural products worldwide. In the US, it is used primarily on corn, sorghum, macadamia nuts and sugarcane, and is applied most heavily in the Midwest. Atrazine is also used on conifer forests; Christmas tree farms; sod farms; golf courses and residential lawns primarily in Florida and the Southeast. Human and wildlife exposure to this herbicide and its metabolites is primarily through run-off during peak farming season and occupational exposure to workers. Levels of atrazine in drinking water supplies have been found to exceed maximal contaminant levels in both the US and Europe (Kello 1989), and atrazine has been banned in several European countries for this reason (Ackerman 2007).

Not surprisingly, atrazine has been widely studied and has been shown by a variety of methods to affect reproductive capacity in both males and females of several species. The exposure and human and environmental health risks of atrazine are also well characterized (Gammon et al. 2005). Atrazine is widely monitored in drinking water supplies, and a variety of mitigation measures are in place to ameliorate risks associated with food, drinking water, workers and residential use. As part of the 2003 registration review, recommendations were made for further testing particularly with respect to carcinogenicity, effects on amphibian endocrinology, and endocrine disrupting potential in general (Environmental Protection Agency 2006). Many studies investigating each of these areas have been performed as summarized below. As a result, all of the animal studies in the EDSP Tier 1 have been performed at least once using recent test guidelines; for some test methods, atrazine was assessed as part of the validation exercise. Therefore, no further testing of atrazine is warranted to fulfill the information requirements for this DCI.

A. Interim Registration Eligibility Decision and Follow-up, 2003 – 2009

In October 2003, EPA issued an Interim Reregistration Eligibility Decision (IRED) for atrazine (Environmental Protection Agency 2006). EPA considered atrazine and structurally-related members of the chlorinated triazine class of pesticides, including simazine, propazine, and their three chlorinated degradates, share a common neuroendocrine mechanism of toxicity which results in both reproductive and

developmental consequences and therefore is issuing tolerances based on a cumulative risk assessment that was completed in 2006.

The atrazine toxicity database is extensive (see list of tests performed for 2003 IRED, **Table 3**). The Agency has reviewed these toxicity studies and has a stated “high degree of confidence in the scientific quality of the toxicity studies conducted with atrazine.” Special studies examining the toxicology of atrazine have been performed by the registrant in addition to the required guideline studies. Additionally, *EPA's National Health and Environmental Effects Laboratory (NHEERL) has performed studies investigating atrazine's neuroendocrine mode of action and related reproductive and developmental effects.*

According to the EPA (Environmental Protection Agency 2006):

“Atrazine is practically non-toxic to slightly toxic to birds and mammals, and relatively non-toxic to honey bees. Atrazine is slightly to moderately toxic to freshwater fish and slightly to highly toxic to freshwater invertebrates. Atrazine is slightly to moderately toxic to estuarine/marine fish and slightly to very highly toxic to estuarine/marine invertebrates.

“Change in hormone levels is the most sensitive health effect observed in an extensive battery of atrazine toxicity tests. In other words, if the Agency's standard is protective of hormonal effects, it will protect against all other effects that occur at higher levels. The Agency's 2003 risk assessment supporting the re-registration of atrazine incorporates standard safety factors to ensure protection of public health, as well as an additional safety factor to ensure further protection for children.

“As a result, EPA's risk assessment includes a 300-fold margin of safety to help ensure that an exposure will not affect hormone levels, and a 1000-fold margin of safety to help protect against long-term or chronic effects. In other words, the exposure that the Agency allows under is at least 300 to 1000 times more protective than the level where the Agency saw no adverse effects in the most sensitive animal species tested. EPA applies these additional safety factors as a precaution to protect the public health of all consumers in the United States.”

Cancer risk: There has been a long-standing concern of cancer risks associated with atrazine exposure for both workers and consumer exposure via food. Atrazine has therefore been widely studied for its carcinogenic potential in rodents, and this data was reviewed before and during the 2003 re-registration review. A FIFRA Scientific Panel (SAP) concluded that “there are considerable differences between hypothalamic-pituitary-ovarian function in rats and humans, and the effect of aging on the function of the axis also is quite dissimilar. *Therefore, it is unlikely that the mechanism by which atrazine induces mammary gland tumors in female SD rats could be operational in humans.*” In its 2003 review, which included epidemiological studies of workers, EPA found that atrazine is “not likely to be carcinogenic in humans” and that “there is a reasonable certainty of no harm from exposure to atrazine so far as cancer risk is concerned.” In an update on the ongoing evaluation of cancer risk due to atrazine

exposure *in July 2009, EPA reiterated its finding that “atrazine is not likely to cause cancer in humans”* and pledged to sponsor epidemiological studies through the National Cancer Institute (NCI) to evaluate the potential for any association between atrazine exposure to people and cancer, even though rigorously conducted animal studies show that this result is unlikely. Other regulators (IARC 1999; European Union 2000; United Kingdom Pesticide Directorate 2000; APVMA 2004) have also concluded that the mammary tumor response observed in female SD rats was not relevant to humans because of differences between species in the mechanisms of neuroendocrine aging of the HPG axis.

While the carcinogenicity potential of atrazine in humans is low, it is through the related mechanism of LH suppression that the triazine pesticides are hypothesized to exert endocrine effects; this hypothesis has been studied in great depth (see below).

Potential effects of atrazine on amphibian endocrinology and development: Because of the lack of reproducibility across studies and an absence of a dose-response relationship in the data available in June, 2003, the Agency determined that it did not have sufficient information to make a determination and requested additional data to reduce uncertainty regarding the potential risk to amphibians. After reviewing additional, newly-generated data, *EPA concluded in 2007 that “atrazine does not adversely affect amphibian gonadal development based on a review of laboratory and field studies, including studies submitted by the registrant and studies published in the scientific literature. At this time, EPA believes that no additional testing is warranted to address this issue.”*

B. Comprehensive Review of Atrazine Planned

In October, 2009, EPA announced plans for a new comprehensive evaluation of atrazine. The purpose of this evaluation is to examine data regarding carcinogenicity and other human health effects of atrazine, including data generated since 2003 from laboratory animal and human epidemiology studies. In 2010, EPA plans to convene two peer reviews of these evaluations.

II. Existing Toxicological Data Related to Endocrine Disruption

The effect of atrazine on vertebrate development and reproduction has been widely studied for over 15 years, as has atrazine’s mechanism of action. In female rodents, Atrazine causes a delay in puberty, disrupts cyclicity, augments the onset of mammary tumors and accelerates reproductive aging. These toxic effects can be tied to a common mechanism of suppression of luteinizing hormone (LH) excretion caused by direct suppression of gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Cooper et al. 2000; Cooper et al. 2007). Consistent with this mode of action, pregnancy loss is seen in atrazine-treated dams only during the LH-sensitive period of pregnancy

(Narotsky et al. 2001). Atrazine appears to mediate its hormonal effects indirectly through the central nervous system.

A. Assessment of estrogenic activity

In both *immature and ovariectomized adult uterotrophic studies*, atrazine failed to demonstrate estrogenic activity but did demonstrate weak anti-estrogenic activity when co-administered with 17 β -estradiol (Tennant et al. 1994; Yamasaki et al. 2000). In *pubertal studies* (carried out as part of the validation studies for the protocol used in Tier 1), atrazine delays vaginal opening and alters estrous cyclicity at 50 mg/kg/day and above [no observed adverse effect level (NOAEL) of 25 mg/kg/day] (Laws et al. 2000). However, this effect has been shown to be a delay rather than a block in sexual maturation, as the animals recover at later developmental stages, again with a NOAEL between 10 and 30 mg/kg/day (Ashby et al. 2002). Interestingly, this study also found that developing rats were relatively insensitive to the effects of atrazine compared to adults.

Atrazine has consistently failed to activate estrogen-dependent reporters *in vitro* in estrogen-dependent expression systems (Eldridge et al. 2008). In relatively recent studies, atrazine failed to stimulate estrogen dependent MCF-7 (Fukamachi et al. 2004) or MtT/E-2 (Fujimoto 2003) cell proliferation. Atrazine also failed to induce estrogen receptor dependent transcription in T47D (Legler et al. 2002) or yeast (O'Connor et al. 2000) cells. A recent review of binding data also concludes that atrazine does not bind the estrogen receptor except at extremely high concentrations (Cooper, Laws et al. 2007).

Exposure of alligator eggs to atrazine resulted in no significant effects on gender differentiation, concentrations of 17 β -estradiol and testosterone or aromatase activity in gonad development (Crain et al. 1997).

A recent review of studies assessing the estrogenic potential of atrazine (Eldridge, Stevens et al. 2008) concludes:

“In summary, from these *in vivo* studies, which employed a wide variety of well-recognized, standard, and specific biological responses to estrogen, it can be concluded that atrazine does not elicit estrogen-like responses, even at dose levels up to a million-fold greater than the minimally effective estrogen dose. ***These results support the conclusion that atrazine is not an estrogen receptor agonist.***”

“In some of the previously described models, however, high doses of atrazine appeared to inhibit or reduce the response to estrogen. This “inhibition” typically occurs at atrazine doses near to, or greater than, the MTD, and at levels

several orders of magnitude greater than the amount of estrogen required to initiate the response. Therefore, one can conclude, from the foregoing review, that ***atrazine antagonism of estrogen-mediated responses in vivo is either nonexistent or extremely weak, and is unlikely to be relevant to man under conditions of potential human exposure.***”

The estrogenic potential of atrazine has been studied in depth, using assays included in the Tier 1 as well as many other in vitro and in vivo assays and the data are conclusive with regard to its activity. Atrazine causes a delay but not permanent block in the onset of puberty in females as well as early reproductive senescence. The NOAEL for these effects is 10 – 30 mg/kg/day. Atrazine does not act through an estrogen-receptor mediated pathway but by direct inhibition of release of GnRH from the hypothalamus. ***Therefore, no additional testing of atrazine for estrogenic activity is warranted.***

B. Assessment of androgenic activity

Atrazine does not appear to have androgenic activity in adult male rodents, except perhaps at very high doses: 200 mg/kg/day caused transient increase in testes size followed by a longer-term decrease, as well as an increase in adrenal size and alterations in steroid hormone levels but no affect on androgen receptor (AR) levels (Victor-Costa et al. 2010). However, an assessment of atrazine using the ***castrate version of the Hershberger*** (following the same protocol as included in the Tier 1 battery) showed no androgenic agonist or antagonist activity of atrazine exposure, nor did atrazine bind to the human recombinant AR (Yamasaki et al. 2004).

However, in developing rodents, similar to the case in females, atrazine causes delayed onset of puberty in males with decreased sex organ weights at high doses (Stoker et al. 2000; Trentacoste et al. 2001; Friedmann 2002). In ***male pubertal*** studies, atrazine caused delay in puberty and reproductive tract development (LOAEL 12.5 mg/kg/day, NOAEL 6.25 mg/kg/day) as well as a significant but variable decrease in serum and testicular testosterone at doses of 100–200 mg/kg/day (Stoker, Laws et al. 2000) and its three chlorinated metabolites appear to have the same effects at similar concentrations (Stoker et al. 2002). Similarly, atrazine applied by gavage at 50 mg/kg/day reduced significantly the serum and intra-testicular testosterone levels, both acutely (from pnd 46 to 48) and chronically (from pnd 22 to 48) (Friedmann 2002).

Similar effects were seen in recent studies looking at early developmental exposure. Exposure during gestation and early postpartum (via the mother’s milk) to atrazine at 100 mg/kg/day resulted in delay of preputial separation and affected the prostate in adults (Rayner et al. 2007). Exposure to atrazine during gestation from PND 14 – parturition resulted in decreased pup survival (10 mg/kg/day and above), decreased anogenital distance (75 mg/kg/day and above) and delayed preputial separation (at 50 mg/kg/day

and above) (Rosenberg et al. 2008). Atrazine exposure did not affect testosterone levels in the testes of the newborn pups' however, serum testosterone levels were significantly reduced at PND 60 (50 mg/kg/day and higher). According to the authors, ***“These results, taken together, are suggestive of anti-androgenic effects of gestational atrazine exposure on male offspring, though these effects occur at doses that are unlikely to be experienced under any but experimental conditions.”***

C. Steroidogenesis

The effect of atrazine on steroid hormone synthesis has been widely studied both *in vivo* and *in vitro*. A recent study of *ex vivo* Leydig cells following peripubertal exposure showed that atrazine exposure decreased expression of several genes responsible for steroidogenesis at doses of 50 mg/kg/day and higher, which is likely to be the underlying cause of the decrease in testosterone seen *in vivo* (Pogrmic et al. 2009). ***Atrazine was tested as part of the development of the Tier 1 H295R assay*** (Higley et al. 2010). In this assessment, atrazine was found not to affect aromatase activity directly, but affected hormone production and enzyme profiles in a pattern similar to forskolin.

D. Assessment of thyroid hormone activity

No effect on thyroid histopathology or hormone levels has been detected in any of the ***in vivo male or female pubertal assays*** (Laws, Ferrell et al. 2000; Stoker, Laws et al. 2000; Stoker, Guidici et al. 2002; Laws et al. 2003).

E. Amphibians and fish

In addition to the reviews undertaken by the EPA during re-registration, there are numerous recent publications describing the effects of atrazine on development and reproduction of amphibians and fish. Since the results of these studies vary considerably, it is difficult to discern a consistent trend with regard to the qualitative and quantitative effects of atrazine on development and reproduction of aquatic vertebrates. A recent meta-analysis was performed to extract trends from existing literature and found some consistencies for freshwater vertebrates, for example: atrazine consistently reduced growth rates, had variable effects on timing of metamorphosis that were often non-monotonic, reduced immunity, induced diverse morphologic gonadal abnormalities associated with modified levels of sex hormones; however, in no study did atrazine affect levels of vitellogenin, suggesting that atrazine does not have estrogenic properties in fish (Rohr and McCoy 2010).

Although some reports have suggested atrazine affects sexual development and gonadal differentiation in *Xenopus laevis* (Hayes et al. 2002; Hayes et al. 2006), other studies did not find significant effects on developing *Xenopus* (Carr et al. 2003; Oka et al. 2008). The result reported by Carr et al. (2003) was confirmed in a larger study conducted concurrently in two laboratories (Kloas et al. 2009). In this study, ***Xenopus laevis exposed to atrazine at concentrations of 0.01, 0.1, 1.0, 25, or 100 ppb from day 8 post-***

fertilization until the completion of metamorphosis demonstrated no effects on any developmental or gonadal parameters. In contrast, estradiol, administered under similar conditions as a control at a concentration of 0.2 ppb resulted in a significant increase in larvae with female or mixed sex gonads, compared to untreated controls.

Atrazine had no effect on a number of reproductive parameters when tested in fathead minnow in the short-term reproduction assay (Bringolf et al. 2004). Nor did atrazine exposure affect vitellogenin in goldfish or carp or induce vitellogenin messenger RNA in zebrafish [reviewed by (Eldridge et al. 2008)].

F. Birds

Atrazine exposure had no significant affect on uterine weight or pituitary LH release in quail (Wilhelms et al. 2006), confirming and expanding earlier studies in which atrazine that showed the absence of estrogen-like effects in the maturing reproductive tracts of male quail administered up to 1000 ppm atrazine (Wilhelms et al. 2005).

III. Summary and Recommendations

Atrazine delays puberty and sexual development in both male and female rodents and has long-term effects in adult male testes; this effect is an indirect affect on the endocrine system through the central nervous system. For most endpoints included in the Tier 1 tests, LOAEL and/or NOAELs have been established. Atrazine does not affect thyroid hormone-dependent processes in rodent or in amphibians (*Xenopus laevis*). Atrazine does not appreciably affect development or sexual differentiation in amphibians or fish, particularly as assessed by protocols similar to the amphibian metamorphosis or fish short-term reproduction Tier 1 assays. Although not addressed in these comments, atrazine has also been tested in a number of estrogen receptor binding and transcriptional activation assays both *in vitro* and *in vivo*; there is no evidence that atrazine binds or activates the estrogen receptor (Eldridge, Stevens et al. 2008). Likewise, there is no evidence that atrazine affects AR binding or activation.

Atrazine has been thoroughly tested in a wide range of vertebrate species using a variety of methods, including protocols similar if not identical to those required under Tier 1 of the EDSP as well as several tests similar to those proposed for Tier 2, including the rodent 2-generation reproductive toxicity test as part of registration and re-registration. In addition, all of the available information regarding atrazine is scheduled to undergo review by EPA in 2010. Therefore there is no conceivable justification for further testing of atrazine as part of the EDSP.

Table 3: Tests Performed as Part of Atrazine IRED 2003

OPPTS Guideline	Study Title
Exotoxicity Data	
850.2200	Avian Dietary Toxicity
850.2300	Avian Reproduction
850.1075	Fish Acute Toxicity - Freshwater
850.1010	Acute Aquatic Invertebrate Toxicity
850.1075	Acute Estuarine/Marine Toxicity - Fish
850.1025	Acute Estuarine/Marine Toxicity - Invertebrate (Mollusk)
	Estuarine/Marine Toxicity - Invertebrate (Shrimp)
850.1400	Early Life-Stage Fish (Freshwater)
	Early Life-Stage Fish (Marine)
850.1300	Life-Cycle Aquatic Invertebrate
850.1350	Life-Cycle Marine Invertebrate
850.1500	Life-Cycle Fish
Mammalian Toxicology	
870.1100	Acute Oral - Rat
870.1200	Acute Dermal - Rabbit
870.1300	Acute Inhalation - Rat
870.2400	Primary Eye Irritation - Rabbit
870.2500	Primary Dermal Irritation - Rabbit
870.2600	Dermal Sensitization
870.3100	90-Day Feeding
870.3200	21-Day Dermal Toxicity - Rat
870.3700	Developmental Toxicity
870.3800	Reproduction and Fertility Effects - 2 Generation Repro
870.4300	Chronic Feeding Toxicity - Rodent Combined Chronic Toxicity/Carcinogenicity
870.4100	Chronic Feeding Toxicity - Non-Rodent (dog)
870.4200	Oncogenicity - Rat
870.4200	Oncogenicity - Mouse
non-guideline	Special Studies - Neurotoxicity
870.5100	Bacterial Reverse Mutation Assay
870.5385	Micronucleus Assay
870.5450	Rodent Dominant Lethal Assay
870.5550	UDS Assay
870.7485	General Metabolism
870.7600	Dermal Absorption
non-guideline	Special Studies - Assays of Direct Estrogenic Activity of Triazines

non-guideline	Special Study - Estrous Cycle Alterations and LH Surge Attenuation - Method Validation
non-guideline	Special Study - Estrous Cycle Alterations and LH Surge Attenuation - Pilot
non-guideline	Special Study - Estrous Cycle Alterations and LH Surge Attenuation - 28-day
non-guideline	Special Study - Estrous Cycle Alterations and LH Surge Attenuation - 6-month
non-guideline	Special Study - Hormone and Estrous Cycle Measurements in SD Rats
non-guideline	Special Study - Mammary Gland and Ovarian Histomorphology in SD Rats
non-guideline	Special Study - Two-Year Bioassay in F-344 Rats
non-guideline	Special Study - Hormone and Estrous Cycle Measurements in F-344 Rats
non-guideline	Special Study - Mammary Gland and Ovarian Histomorphology in F-344 Rats
non-guideline	Special Study - Two-year Bioassay with the SD Strain of Rate
non-guideline	Special Study - Tumor Incidence in Ovariectomized (OVX) vs Intact Animals
non-guideline	Special Study - Sexual Maturation
non-guideline	Long-Term Estrous Cycle Measurements
non-guideline	Direct Comparison of LH Surge Attenuation of Atrazine, Simazine, and Dact
TOXICOLOGY - Degradate DACT	
870.1100	Acute Oral - Rat
870.3100	Subchronic Oral Toxicity in Rats
870.3150 870.4100	Subchronic & Chronic Oral Toxicity in Dogs
870.3700	Developmental Toxicity in Rats
870.5100	Mutagenicity Study - Bacterial Reverse Mutation
870.5550	Mutagenicity Study - UDS Assay
TOXICOLOGY - Degradate Desisopropyl Atrazine	
870.1100	Acute Oral - Rat
870.3100	Subchronic Oral Toxicity in Rats
870.3150	Subchronic Oral Toxicity in Dogs
870.3700	Developmental Toxicity in Rats
870.5100	Mutagenicity Study - Bacterial Reverse Mutation Assay
870.5385	Mutagenicity Study - Mammalian Bone Marrow Chromosome Aberration Test
870.5550	Mutagenicity Study - UDS Assay
TOXICOLOGY - Degradate Deethyl Atrazine	
870.1100	Acute Tox - Rat
870.3100	Subchronic Oral Toxicity in Rats
870.3150	Subchronic Oral Toxicity in Dogs
870.3700	Developmental Toxicity in Rats
870.5100	Mutagenicity Study - Bacterial Reverse Mutation Assay
870.5385	Mutagenicity Study - Mammalian Bone Marrow Chromosome Aberration Test
870.5550	Mutagenicity Study - UDS Assay

TOXICOLOGY - Degradate Hydroxyatrazine	
870.3100	Subchronic Oral Toxicity in Rats
870.3700	Developmental Toxicity in Rats
870.4100 870.4300	Chronic Toxicity - Rat
870.5100	Mutagenicity Study - Bacterial Reverse Mutation Assay
870.5385	Mutagenicity Study - Mammalian Bone Marrow Chromosome Aberration Test
870.5550	Other Genetic Effects
EPIDEMIOLOGY	
non-guideline	Cancer Epidemiologic Studies - Submitted to EPA
non-guideline	Cancer Epidemiologic Studies - Other Published Literature

IV: References

- Ashby, J., H. Tinwell, J. Stevens, T. Pastoor and C. B. Breckenridge (2002). "The Effects of Atrazine on the Sexual Maturation of Female Rats " Regulatory Toxicology and Pharmacology **35**(3): 468-473.
- Bringolf, R. B., J. B. Belden and R. C. Summerfelt (2004). "Effects of atrazine on fathead minnow in a short-term reproduction assay." Environ Toxicol Chem **23**(4): 1019-1025.
- Carr, J. A., A. Gentles, E. E. Smith, W. L. Goleman, L. J. Urquidi, K. Thuett, R. J. Kendall, J. P. Giesy, T. S. Gross, K. R. Solomon and G. Van Der Kraak (2003). "Response of larval *Xenopus laevis* to atrazine: assessment of growth, metamorphosis, and gonadal and laryngeal morphology." Environ Toxicol Chem **22**(2): 396-405.
- Cooper, R. L., S. C. Laws, P. C. Das, M. G. Narotsky, J. M. Goldman, E. Lee Tyrey and T. E. Stoker (2007). "Atrazine and reproductive function: mode and mechanism of action studies." Birth Defects Res B Dev Reprod Toxicol **80**(2): 98-112.
- Cooper, R. L., T. E. Stoker, L. Tyrey, J. M. Goldman and W. K. McElroy (2000). "Atrazine disrupts the hypothalamic control of pituitary-ovarian function." Toxicol Sci **53**(2): 297-307.
- Crain, D. A., L. J. Guillette, Jr., A. A. Rooney and D. B. Pickford (1997). "Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants." Environ Health Perspect **105**(5): 528-533.
- Eldridge, J. C., J. T. Stevens and C. B. Breckenridge (2008). Atrazine interaction with estrogen expression systems. Rev Environ Contam Toxicol. **196**: 147-160.
- Eldridge, J. C., J. T. Stevens and C. B. Breckenridge (2008). "Atrazine interaction with estrogen expression systems." Rev Environ Contam Toxicol **196**: 147-160.

- Environmental Protection Agency, U. (2006). "Decision Documents for Atrazine."
Retrieved 14 Jan 2010, 2010, from
http://www.epa.gov/oppsrrd1/REDS/atrazine_combined_docs.pdf.
- Friedmann, A. S. (2002). "Atrazine inhibition of testosterone production in rat males following peripubertal exposure." *Reprod Toxicol* **16**(3): 275-279.
- Fujimoto, N. (2003). "Effects of environmental estrogenic compounds on growth of a transplanted estrogen responsive pituitary tumor cell line in rats." *Food Chem Toxicol* **41**(12): 1711-1717.
- Fukamachi, K., B. S. Han, C. K. Kim, N. Takasuka, Y. Matsuoka, E. Matsuda, T. Yamasaki and H. Tsuda (2004). "Possible enhancing effects of atrazine and nonylphenol on 7,12-dimethylbenz[a]anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats." *Cancer Sci* **95**(5): 404-410.
- Gammon, D., C. Aldous, W. J. Carr, J. Sanborn and K. Pfeifer (2005). "A risk assessment of atrazine use in California: human health and ecological aspects." *Pest Manag Sci* **61**(4): 331-355.
- Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart and A. Vonk (2002). "Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses." *Proc Natl Acad Sci U S A* **99**(8): 5476-5480.
- Hayes, T. B., A. A. Stuart, M. Mendoza, A. Collins, N. Noriega, A. Vonk, G. Johnston, R. Liu and D. Kpodzo (2006). "Characterization of atrazine-induced gonadal malformations in African clawed frogs (*Xenopus laevis*) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17beta-estradiol): Support for the demasculinization/feminization hypothesis." *Environ Health Perspect* **114 Suppl 1**: 134-141.
- Higley, E., J. Newsted, X. Zhang, J. Giesy and M. Hecker (2010). "Assessment of chemical effects on aromatase activity using the H295R cell line." *Environ Sci Pollut Res Int*.
- Kloas, W., I. Lutz, T. Springer, H. Krueger, J. Wolf, L. Holden and A. Hosmer (2009). "Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*?" *Toxicol Sci* **107**(2): 376-384.
- Laws, S. C., J. M. Ferrell, T. E. Stoker and R. L. Cooper (2003). "Pubertal development in female Wistar rats following exposure to propazine and atrazine biotransformation by-products, diamino-S-chlorotriazine and hydroxyatrazine." *Toxicol Sci* **76**(1): 190-200.
- Laws, S. C., J. M. Ferrell, T. E. Stoker, J. Schmid and R. L. Cooper (2000). "The effects of atrazine on female wistar rats: an evaluation of the protocol for assessing pubertal development and thyroid function." *Toxicol Sci* **58**(2): 366-376.

- Legler, J., M. Dennekamp, A. D. Vethaak, A. Brouwer, J. H. Koeman, B. van der Burg and A. J. Murk (2002). "Detection of estrogenic activity in sediment-associated compounds using in vitro reporter gene assays." Sci Total Environ **293**(1-3): 69-83.
- Narotsky, M. G., D. S. Best, D. L. Guidici and R. L. Cooper (2001). "Strain comparisons of atrazine-induced pregnancy loss in the rat." Reprod Toxicol **15**(1): 61-69.
- O'Connor, J. C., D. R. Plowchalk, C. S. Van Pelt, L. G. Davis and J. C. Cook (2000). "Role of prolactin in chloro-S-triazine rat mammary tumorigenesis." Drug Chem Toxicol **23**(4): 575-601.
- Oka, T., O. Tooi, N. Mitsui, M. Miyahara, Y. Ohnishi, M. Takase, A. Kashiwagi, T. Shinkai, N. Santo and T. Iguchi (2008). "Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*." Aquat Toxicol **87**(4): 215-226.
- Pogrmic, K., S. Fa, V. Dakic, S. Kaisarevic and R. Kovacevic (2009). "Atrazine oral exposure of peripubertal male rats downregulates steroidogenesis gene expression in Leydig cells." Toxicol Sci **111**(1): 189-197.
- Rayner, J. L., R. R. Enoch, D. C. Wolf and S. E. Fenton (2007). "Atrazine-induced reproductive tract alterations after transplacental and/or lactational exposure in male Long-Evans rats." Toxicol Appl Pharmacol **218**(3): 238-248.
- Rohr, J. R. and K. A. McCoy (2010). "A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians." Environ Health Perspect **118**(1): 20-32.
- Rosenberg, B. G., H. Chen, J. Folmer, J. Liu, V. Papadopoulos and B. R. Zirkin (2008). "Gestational exposure to atrazine: effects on the postnatal development of male offspring." J Androl **29**(3): 304-311.
- Stoker, T. E., D. L. Guidici, S. C. Laws and R. L. Cooper (2002). "The effects of atrazine metabolites on puberty and thyroid function in the male Wistar rat." Toxicol Sci **67**(2): 198-206.
- Stoker, T. E., S. C. Laws, D. L. Guidici and R. L. Cooper (2000). "The effect of atrazine on puberty in male wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function." Toxicol Sci **58**(1): 50-59.
- Tennant, M. K., D. S. Hill, J. C. Eldridge, L. T. Wetzel, C. B. Breckenridge and J. T. Stevens (1994). "Chloro-s-triazine antagonism of estrogen action: limited interaction with estrogen receptor binding." J Toxicol Environ Health **43**(2): 197-211.
- Trentacoste, S. V., A. S. Friedmann, R. T. Youker, C. B. Breckenridge and B. R. Zirkin (2001). "Atrazine effects on testosterone levels and androgen-dependent reproductive organs in peripubertal male rats." J Androl **22**(1): 142-148.
- Victor-Costa, A. B., S. M. Bandeira, A. G. Oliveira, G. A. Mahecha and C. A. Oliveira (2010). "Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine." Reprod Toxicol.

- Wilhelms, K. W., S. A. Cutler, J. A. Proudman, L. L. Anderson and C. G. Scanes (2005). "Atrazine and the hypothalamo-pituitary-gonadal axis in sexually maturing precocial birds: studies in male Japanese quail." Toxicol Sci **86**(1): 152-160.
- Wilhelms, K. W., S. A. Cutler, J. A. Proudman, R. V. Carsia, L. L. Anderson and C. G. Scanes (2006). "Lack of effects of atrazine on estrogen-responsive organs and circulating hormone concentrations in sexually immature female Japanese quail (*Coturnix coturnix japonica*)." Chemosphere **65**(4): 674-681.
- Yamasaki, K., M. Sawaki, S. Noda, T. Muroi, S. Takakura, H. Mitoma, S. Sakamoto, M. Nakai and Y. Yakabe (2004). "Comparison of the Hershberger assay and androgen receptor binding assay of twelve chemicals." Toxicology **195**(2-3): 177-186.
- Yamasaki, K., M. Sawaki, Shuji Noda, T. Muroi and A. Maekawa (2000). "Immature Rat Uterotrophic Assay of Diethylstilbestrol, Ethynyl Estradiol and Atrazine." J Toxicol Pathol **13**: 145-149.

2,4-dichlorophenoxyacetic acid (2,4-D), CAS number 94-75-7

Test order numbers EDSP-080803-5 through 14

Test order date: October 29, 2009

I. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) is an herbicide that is used post-emergence for control of broadleaf weeds. 2,4-D has been registered in the United States since 1948, and it is one of the best studied of all agricultural chemicals. A 1978 review stated that more than 40,000 scientific articles and technical reports addressing 2,4-D had been published at that time (Munro, 1992). Several data call-ins (DCI) have already been issued for 2,4-D. In 1980, registrants of 2,4-D products were required to submit studies on acute toxicity, oncogenicity, reproductive effects, teratogenicity, neurotoxicity and metabolism. The registrants jointly entered into an agreement to produce the requested data as the Industry Task Force on 2,4-D Research Data (ITF). Since that time, the ITF has conducted, sponsored, or otherwise reviewed hundreds of additional scientific studies of the safety of 2,4-D (ITF, 2009).

II. Existing Toxicological Data Related to Endocrine Disruption

2,4-D has low acute toxicity via the oral, dermal and inhalation routes of exposure. A series of GLP 90-day dietary toxicity studies in rats established the NOEL for 2,4-D as 15 mg /kg/day (Charles et. al., 1996). With regard to endocrine disrupting potential, only minimal or no effects were observed in estrogen or androgen sensitive tissues in these studies, and these effects were restricted to high doses which exceeded either the maximum tolerated dose or the renal clearance capacity. With regard to thyroid endpoints in these studies, such as those addressed in Tier 1 pubertal rat tests, thyroid weights increased in the highest dose groups, but histologic evaluation revealed only non-significant increases in parafollicular cell nodular hyperplasia. ***No evidence of treatment-related effects was observed at dose levels below 75 mg/kg/day.*** Similarly, decreased serum levels of T4 and/or T3 were also observed only at doses that exceeded renal clearance.

More recently, members of the ITF investigated the potential developmental toxicity of 2,4-D in a series of ***eight GLP studies in rats and seven in rabbits*** (Charles et.al., 2001). In rats, litter sizes, resorption rates, and fetal sex ratios were unaffected by treatment at doses up to 90 mg/kg/day (acid equivalent) and the overall developmental NOEL for all rat studies was approximately 30 mg/kg/day. Significantly decreased fetal body weights, slightly delayed skeletal ossification and the presence of extra ribs were observed in rats only at maternally toxic dose levels in excess of 90 mg/kg/day. In rabbits, maternal

reproductive measures as well as embryonic and fetal development were essentially unaffected even at maternally toxic doses. Since no adverse fetal effects were observed at dose levels that did not also produce maternal toxicity or exceed renal clearance, ***the authors concluded that the developing rat and rabbit fetus were not uniquely sensitive to 2,4-D.***

In 2007, a DCI was issued for a two-generation reproduction study that specifically required examination of thyroid, gonadal, reproductive and other endocrine-sensitive endpoints. The ITF has worked closely with the EPA to design a state-of-the-art extended one-generation reproduction study with 2,4-D to address these concerns (ITF, 2009). ***This study will provide an apical assessment of the potential endocrine properties of 2,4-D in rats, supplanting any mammalian toxicity information to be gained from the EDSP. The in-life phase of this study has been completed, and the ITF anticipates submitting a report to the EPA “in the not-too-distant future” (ITF, personal communication).***

In addition to these definitive studies directly addressing mammalian endpoints to be assessed in EDSP Tier 2 assays, 2,4-D has been found to be practically non-toxic to freshwater or marine fish and birds on an acute basis (EPA, 2005). The chronic toxicity endpoint in fish is based on larval length and survival. The avian chronic endpoint is based on the number of eggs cracked and decreased number of eggs laid. In its comments on The Natural Resources Defense Council’s *Petition to Revoke All Tolerances and Cancel All Registrations for the Pesticide 2,4-D*, the ***ITF presents a table listing 11 in vitro assays indicating that 2,4-D is negative for estrogenicity (ITF, 2009).*** These include assays measuring human estrogen receptors (ER) alpha and beta transcriptional activation, MCF7 cell proliferation, and rat, alligator and trout ER binding. Further, *in ovo* exposure of alligator eggs to a range of 2,4-D concentrations had no effect in the hatchlings to the estrogen-sensitive endpoints of production of females at male-determinant egg incubation temperatures, gonadal and reproductive histology and hepatic or gonad adrenalmesonephros aromatase activity (Spiteri et.al, 1999, cited in ITF, 2009).

Human studies have also addressed the potential reproductive and developmental toxicity of 2,4-D. Vietnam veterans’ risks for fathering babies with major structural birth defects were assessed using a case-control study (Erickson JD et al., 1984). The authors concluded Vietnam veterans in general as well as Vietnam veterans who had greater estimated opportunities for Agent Orange exposure did not have an increased risk of fathering babies with defects. While initial epidemiologic studies by Garry et al. (1996) indicated an increased frequency of birth defects among pesticide applicators and the general population in an agricultural region of Minnesota, in more recent reports, these authors concluded that a more detailed cross-sectional analysis of this area showed ***no***

statistically significant correlations between 2,4-D use and excess adverse birth or neurodevelopmental effects (Garry et.al., 2002, cited in ITF, 2009).

III. Summary and Recommendations

In summary, 2,4-D is an extremely well-studied chemical. Its endocrine disrupting potential has already been addressed in mammalian subchronic, reproductive and developmental studies as well as in numerous *in vitro* assays, in chronic studies in birds, fish and reptiles and in epidemiologic studies. ***Further, a study designed in consultation with the EPA to definitively address remaining endocrine-sensitive endpoints is nearing completion. There is no need for further testing under the EDSP.***

IV. References

- Charles, J.M., Cunny, H.C., Wilson R.D., and Bus, JS. 1996. Comparative Subchronic Studies on 2,4-Dichlorophenoxyacetic Acid, Amine and Ester in Rats. *Fund. Appl. Toxicol.* 33: 161-165.
- Charles, J.M., Hanley, T.R. Jr., Wilson, R.D., van Ravenzwaay, B., Bus, J.S. 2001. Developmental toxicity studies in rats and rabbits in 2,4-dichlorophenoxyacetic acid and its forms. *Tox.Sci.* 60: 121-131.
- Erickson J.D., Mulinare J., McClain P.W., Fitch T.G., James L.M., McClearn A.B., Adams M.J. Jr. 1984. Vietnam veterans' risks for fathering babies with birth defects. *JAMA.* 252(7):903-12.
- Industry Task Force II on 2,4-D Research Data (ITF). 2009. Comments on The Natural Resources Defense Council's Petition to Revoke All Tolerances and Cancel All Registrations for the Pesticide 2,4-D. Docket ID EPA-HQ-OPP-2008-0877. Available at: <http://www.regulations.gov/search/Regs/contentStreamer?objectId=09000064808be666&disposition=attachment&contentType=pdf>.
- Munro, I. C., Carlo, G. L., Orr, J. C., Sund, K. G., Wilson, R. M., Kennepohl, E., Lynch, B. S., Jablinske, M., and Lee, N. L. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. *J. Amer. Coll. Toxicol.* 11: 559-664.
- United States Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances. 2005. Reregistration Eligibility Decision for 2,4-D. Available at: www.epa.gov/oppsrrd1/REDS/24d_red.pdf.

Benfluralin, CAS number 1861-40-1

Test order numbers EDSP-084301-15 - 16

Test order date: October 29, 2009

I. Introduction

Benfluralin is a food use pre-emergent dinitroaniline herbicide first registered in 1970. It is used in a number of settings including residential and commercial turf management, greenhouses, and rights of way. It is also used on some food crops, including lettuce, alfalfa for forage, and some nonbearing nut and fruit trees and vines.

The information available for benfluralin includes a complete standard data package, like most registered pesticides. While it is not considered acutely toxic, it is considered highly toxic to aquatic organisms and has been shown to cause reproductive toxicity in two bird species. In 13- and 52-week dog studies, and in several 90-day rat and mouse studies, the liver was found to be the target organ of most concern; some nephrotoxicity also presented. Increased incidence of thyroid tumors in a two year rat carcinogenicity bioassay¹ raises some concern for disruption of the EAT systems; however a lack of effect in reproductive and developmental guideline studies indicate estrogen or androgen activity is not likely.

Benfluralin has been the subject of several reviews, including a US EPA Reregistration Eligibility Decision in 2004 (EPA 2004), a California EPA DPR summary (CalEPA 2000) and a European Food Safety Authority (EFSA) Peer Review in 2008 (EFSA 2008). Additionally, Benfluralin was assessed through the EPA ToxCastTM program, using a number of binding and reporter assays relevant to the EAT system as well as nuclear receptor assays. It is also possible that registrants have conducted other mechanistic studies to address the appearance of thyroid tumors in rats. Considering this, as well as all existing scientifically relevant information, the data that would be generated from *in vivo* Tier 1 assays on Benfluralin would not add value.

II. Existing Toxicological Data related to endocrine disruption

Results from several studies for benfluralin do not raise concern for estrogenic or androgenic activity in mammals.

In a 2-year cancer bioassay using rats, major effects were noted on kidney structure and function, and thyroid follicular tumors were also present. Thyroid adenomas and carcinomas increased in incidence in males and females at the top two of four doses; however, benfluralin is not thought to be genotoxic. Additional mechanistic data to explain the presence of thyroid tumors does not appear to be publicly available, but may be privately available.

Two teratogenicity studies (one using rabbits and one using rats) and two 2-generation rat reproductive studies--proposed Tier 2 studies--did not indicate any effects related to endocrine modulation by benfluralin (EPA 2004 and EFSA 2006).

According to the EPA's Reregistration Eligibility Decision Factsheet (EPA 2004), Benfluralin is thought to be a reproductive toxicant in birds and acutely toxic to aquatic organisms. Two reproductive studies in Mallard Duck and Bobwhite Quail were available at the time of reregistration; an additional Bobwhite Quail study was requested because a LOAEC could not be established from the first study. A fish life cycle test was also requested. If available, these studies add to the information about the potential for Benfluralin to interact with the EAT system. The chemical will likely be regulated on this data, making it unclear what additional Tier 1 amphibian and fish testing will accomplish.

The EPA's ToxCast™ program profiled 56 of the 73 EDSP Phase I chemicals, including benfluralin, 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-driven assays (Kavlock et al 2009). The advantage of the structure of the ToxCast Program is that connections can rapidly be made between assay result readouts and existing mammalian and ecotoxicity data, which may be directly applicable to determining, for example, the significance of thyroid tumors in the rat study mentioned above.

Preliminary results from Phase I of the entire ToxCast™ program are promising (Judson et al 2010). 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, substances that show the most "activity" across a large number of molecular pathways also inversely correlate with lower effect levels in mammalian studies.

It is this type of coordinated, mechanistic investigation that the Agency should be completing for EDSP chemicals. Once existing data on the Phase I chemicals is gathered, mechanistic *in silico* and *in vitro* investigations should follow, before any vertebrate testing is considered.

III. Summary

Information that is the equivalent of EDSP Teir 2 data is available for benfluralin that indicates no estrogen or androgen activity. There is some indication of interference with thyroid-related processes; however, all of the information presented here, as well as information from registrants, and information from the ToxCast™ program, should be considered before requiring additional *in vivo* testing.

IV. References

Environmental Protection Agency (2004) Benfluralin RED Factsheet. Available at <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100113E.txt>. Accessed February 4, 2010.

- California Environmental Protection Agency Department of Pesticide Regulation (2000) Benfen Summary of Toxicological Data. Available at <http://www.cdpr.ca.gov/docs/toxsums/pdfs/53.pdf>. Accessed February 4, 2010.
- European Food Safety Authority (2008) Conclusion regarding the peer review of the pesticide risk assessment of the active substance benfluralin. Available at <http://www.efsa.europa.eu/en/scdocs/scdoc/127r.htm>. Accessed February 4, 2010.
- Judson et al. (2010) "In Vitro Screening of Environmental Chemicals for Targeted Testing Prioritization – The ToxCast Project" Environmental Health Perspectives {doi:10.1289/ehp.0901392}.
- Kavlock et al. (2009) Biological Profiling of Endocrine Related Effects of Chemicals in ToxCastTM. Poster presentation available at <http://www.epa.gov/NCCT/toxcast/files/summit/40P%20Kavlock%20TDAS.pdf>. Accessed February 4, 2010.
- Additional information gathered from TOXNET database (<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@na+BENFLURALIN>) and EPA ACToR database (<http://actor.epa.gov/actor/faces/ACToRHome.jsp>). Accessed February 4, 2010.

Dimethyl tetrachloroterephthalate (DCPA), CAS number 1861-32-1

Test order numbers EDSP-080803-1 through 4

Test order date: October 29, 2009

I. Introduction

DCPA (dimethyl tetrachloroterephthalate; chlorthal-dimethyl; Dacthal) is a chlorinated benzoic acid herbicide initially registered in 1958 that appears, along with its metabolites, to have low acute and chronic toxicity.¹ While DCPA is not considered environmentally persistent or mobile (with a half life between fifteen and thirty days), its principal metabolite, tetrachloroterephthalic acid (TPA), is considered both persistent and mobile with a tendency to contaminate groundwater wherever DCPA is applied.² 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and hexachlorobenzene (HCB) are known contaminants of industrially synthesized DCPA and are constituent in all DCPA product formulations.^{2,3,4,5,6} While DCPA has been classified by EPA as a Possible Human Carcinogen (Group C), both 2,3,7,8-TCDD and HCB are considered Probable Human Carcinogens (Group B2) and may impact endocrine function themselves, the latter of which is classified as a reproductive hazard in the state of California.²

DCPA has been thoroughly examined in more than a dozen studies involving several species that have not indicated a need for further endocrine-specific testing, and existing epidemiological data does not support a developmental impact for this chemical that has been widely available and in heavy use for more than 40 years. Examining DCPA with the *in vivo* assays planned for EPA's two-tiered Endocrine Disruptor Screening Program (EDSP) testing batteries would duplicate existing multigenerational data and would not modify the chemical's regulation.

II. Existing Toxicological Data

Existing data does not suggest a connection between DCPA exposure at any dose range and possible endocrine disruption. Of the fourteen DCPA studies cited in EPA's DCPA 1998 Reregistration Eligibility Decision (RED) and Integrated Risk Information System (IRIS), *twelve studies conclude that there were no variations, histological or otherwise, that would be considered consistent with any compound effect.*² More than 45 years ago, a study of dogs fed DCPA in the diet at up to 250 milligrams per kilogram bodyweight per day (mg/kg/d) noted that "[t]he organs of the male and female dogs from the three different dosage levels presented histological variations that were not consistent nor indicative of a compound effect."⁷ *A two-generation reproductive toxicity assay* using 280 rats administered doses up to 1,273 mg/kg/d noted that no differences were observed in reproductive performance during the growth phase, mating, gestation and lactation for two generations that each bore two litters.⁸ Two studies examining reproductive and developmental effects of DCPA administered to gestating rats identified

no adverse effects in mothers or pups, and two similar studies using rabbits showed that *"[m]aternal reproductive parameters were not affected by treatment and no embryotoxicity, fetotoxicity or teratogenicity was observed at any dose level tested."*^{9,10,11,12}

Although the specific histological and pathological endpoints included in EDSP Tier 1 and Tier 2 testing are not included in the data from these studies, definitive findings from these studies can be applied to the information requested by the DCI. One study using mice fed DCPA in the diet illustrated that "[n]o treatment-related effects were observed on survival or clinical signs in either sex at any dose level tested" up to the maximum of 1,000 mg/kg/d.¹³ A thirteen week oral study in mice concluded that there were no treatment-related effects of DCPA exposure on survival, body weight, food consumption or other clinical signs in either sex.¹⁴ 36 and 28 day oral dosing studies in rats also demonstrated a lack of treatment-related effects in an array of endpoints that included clinical signs such as organ weights, histopathology, and changes in body weight.^{15,16}

A. Liver toxicity

In the vast majority of these fourteen studies, the only histopathological changes noted among test groups were limited to effects characteristic of upregulated metabolic and excretory functions such as liver hypertrophy and chronic nephropathy.¹⁷ A two year study in 900 mice fed DCPA at doses up to 7,500 mg/kg/d points out that "the only effects observed following exposure to the test material were on the liver."¹⁸ This result is echoed in another study using mice, this time on a thirteen-week scale in which the DCPA dose was as high as 10,000 parts per million (ppm).¹⁹ It has been noted that "chronic nephropathy is a commonly observed progressive lesion in Sprague-Dawley rats," and "exacerbation of this common aging lesion was apparently the main cause of spontaneous death or moribundity" in one DCPA study.¹⁷ Furthermore, many of these clinical signs are similar to those observed in animals dosed with relatively minute amounts of 2,3,7,8-TCDD and HCB, of which the latter is known to concentrate in breast milk and pass from mother to child during nursing.^{4,5,6,20} It is difficult to presume that any observed effect is due solely to the presence of DCPA and not in part to the presence of these much more acutely and chronically toxic contaminants or to inherent biological responses to metabolic activation in certain species.

B. Toxicological data related to endocrine activity

Only two of the fourteen DCPA studies cited in IRIS describe histopathological changes that are superficially associated with possible endocrine disruption, although neither seems to suggest specific treatment-related effects. In one instance, thyroid weights and thyroid hormone profiles were modulated in test rats of both sexes.¹⁷ The authors of this

study note, however, that the changes observed were in fact indirect effects of liver damage and metabolic activity set in motion by large doses of the test compound:

“...thyroid hormones are metabolized by the liver and excreted in the bile. Lower concentrations of the thyroid hormones can result following metabolic activation, which can lead to an increased release of TSH from the pituitary gland, via a feedback mechanism, and stimulation of the follicular cells. This in turn can result in follicular cell hypertrophy and hyperplasia following prolonged stimulation and ultimately follicular cell neoplasms. *The findings in this study with respect to the liver and thyroid suggest a possible indirect effect of Dacthal on the thyroid.*”¹⁷

Therefore, the thyroid effect is not the result of DCPA’s putative endocrine activity, but rather a residual effect of the liver’s upregulated activity. The second study to produce a clinical measure suggestive of endocrine activity was a 1963 study using rats that noted a change in adrenal weights within the test group.²¹ This result, however, has not been replicated in 13 subsequent DCPA-dosing studies in a variety of species, including among the nine other studies in the rat that included DCPA doses within the range of the 1963 study. *Additionally, EPA’s ToxRef Database does not consider results of DCPA multigenerational reproductive toxicity tests to be consistent with endocrine-related organ pathologies.*

C. Epidemiology

Epidemiological studies, while noting the ubiquity of human exposure to DCPA among agricultural communities and other vulnerable populations, have failed to identify effects that would suggest possible endocrine disruption among the exposed.²² With a detailed record of contamination in the homes and cities located in regions of agricultural DCPA application, no adverse reproductive or developmental effects have been noted. One 2009 study of a prospective cohort of pregnant women during gestation suggested that DCPA crosses the placental barrier yet does not affect developmental measures of the child including head circumference, abdominal circumference, or birth weight.²³

III. Summary and Recommendations

Existing data used to satisfy EPA’s 1998 RED requirements have yielded valuable information about DCPA’s toxicity profile while concurrently providing the information sought in EDSP chemical screening and testing. Furthermore, EPA’s plans to screen DCPA for endocrine disruptive potential fail to consider putative roles of known chemical contaminants and metabolites which have an unknown capacity to affect hormone-related systems. To justify further testing, EPA should articulate what

additional information is required to adequately regulate this substance before additional tests using animals are required.

Although current EDSP criteria state that Phase I chemicals are selected based solely on their modes of exposure, EPA has commented that, in the future, it may amend this selection process by considering existing data on known compound effects of chemicals in question. In that context, it is important to note that data from experiments using animals and epidemiological evidence to date have failed to suggest that DCPA has the potential to interfere with endocrine processes. ***More specifically, mammalian multigenerational studies have shown no effect on fertility or reproductive endpoints in a variety of species across a broad exposure range. Over 3,300 animals were killed in the studies cited in IRIS alone, and a general consensus from those studies is that DCPA is not a priority chemical for EDSP.***

As previous multigenerational studies have shown no effect on fertility or reproductive endpoints in a variety of species across a broad exposure range it is unlikely that further testing in Tier 1 assays, whose purpose is to determine which, if any, further testing is warranted, will yield any useful information. We suggest that EPA consider the preponderance of existing animal DCPA toxicity data and adopt an integrated approach to further data collection from human-relevant *in vitro* and epidemiologic sources. Considering the ubiquity of long-term human exposure to this herbicide, we recommend that EPA more closely examine DCPA's influence on human physiology by surveying populations exposed during the compound's almost five decades of registered use.

IV. References

- ¹ U.S. Environmental Protection Agency. Office of Pesticides and Toxic Substances. Office of Pesticide Programs. 1988. Pesticide fact sheet (DCPA). Washington, D.C.
- ² U.S. Environmental Protection Agency. Office of Pesticides and Toxic Substances. 1988. *Guidance for the reregistration of pesticide products containing dimethyl tetrachloroterephthalate (DCPA) as the active ingredient*. Washington, D.C.
- ³ Silbergeld, E.K. and T.A. Gasiewicz. 1989. Commentary: Dioxins and the Ah receptor. *Amer. J. of Industrial Med.* 16:455-474.
- ⁴ Murray, F.J. et al. 1979. Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin in the diet. *Tox. Appl. Pharm.* 50:241.
- ⁵ Nisbet, I. and M. Paxton. 1982. Statistical aspects of three-generation studies of the reproductive toxicity of TCDD and 2,4,5-T. *Am. Statistician* 36(3):290.
- ⁶ U.S. Environmental Protection Agency. 1980. *Dioxins* (600-2-80-197). Washington, D.C.

-
- ⁷ Diamond Alkali Company. 1963. Unpublished study, EPA IRIS Database.
- ⁸ ISK Biotech Corporation (ISK). 1990. MRID No. 41750103, 41905201. HED Doc. No. 008134, 008444. Unpublished study, EPA IRIS Database.
- ⁹ SDS Biotech Corporation (SDS). 1986. MRID No. 00160685. HED Doc. No. 005866, 009515. Unpublished study, EPA IRIS Database.
- ¹⁰ SDS. 1985. MRID No. 00158010. HED Doc. No. 005706. Unpublished study, EPA IRIS Database.
- ¹¹ Fermenta Plant Protection Company (Fermenta). 1989. MRID No. 41054820. HED Doc. No. 008229, 008409. Unpublished study, EPA IRIS Database.
- ¹² Fermenta ASC Corporation. 1990. MRID No. 41349101, 41750102. HED Doc. No. 008373. Unpublished study, EPA IRIS Database.
- ¹³ ISK. 1991. MRID No. 41767901. HED Doc. No. 008366. Unpublished study, EPA IRIS Database.
- ¹⁴ Fermenta. 1986. MRID No. 41064801. HED Doc. No. 008373. Unpublished study, EPA IRIS Database.
- ¹⁵ SDS. 1985. MRID No. 00158010. HED Doc. No. 005706. Unpublished study, EPA IRIS Database.
- ¹⁶ ISK. 1990b. MRID No. 41790901. HED Doc. No. 008408. Unpublished study, EPA IRIS database.
- ¹⁷ ISK 1993. MRID No. 42731001, 42998401. HED Doc. No. 010513. Unpublished study, EPA IRIS Database.
- ¹⁸ Fermenta. 1988. MRID No. 40958701. HED Doc. No. 007250, 008095. Unpublished study, EPA IRIS Database.
- ¹⁹ Fermenta. 1986. MRID No. 41064801. HED Doc. No. 008373. Unpublished study, EPA IRIS Database.
- ²⁰ U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry. 1990. *Toxicological profile for hexachlorobenzene*. Washington, D.C.
- ²¹ Diamond Alkali Company. 1963b. MRID No. 00083577. HED Doc. No. 003299, 005866. Unpublished study, EPA IRIS Database.
- ²² Bradman A et al. Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. *Journal of Exposure Science and Environmental Epidemiology*. (2007) 17, 331–349.
- ²³ Barr D et al. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Science of the Total Environment*. (2009) November 7 electronic publication ahead of print.

Fenbutatin oxide, CAS number 13356-08-6

Test order number EDSP-104601-18

Test order date: October 29, 2009

I. Introduction

Fenbutatin oxide is an aryl organotin acaricide that interferes with oxidative phosphorylation and photophosphorylation. It is a restricted-use pesticide, registered since 1974. It is currently approved for ornamental, agricultural and residential uses. Recent assessments of fenbutatin oxide, including the December 2009 Registration Review and the August 2007 new use assessment on pistachios (USEPA, 2007; DP328390), detail data relevant for both aquatic and mammalian species which call into question the utility of further testing under the Endocrine Disruptor Screening Program. In particular, there are data relevant for potential endocrine effects in fish and mammalian species that could be used to satisfy the five Tier 1 *in vivo* tests in those species.

II. Existing Toxicological Data Related to Endocrine Disruption

Existing data indicate that chronic exposure to freshwater fish (NOAEC = 0.31 µg/L; LOAEC = 0.61 µg/L) results in reductions in larval growth and survival, while fresh water invertebrates (NOAEC = 16 µg/L; LOAEC = 39 µg/L) demonstrate reductions in growth, adult survival, and reproduction (Peck C. et al., 2009). These data should be considered in place of the ecotoxicity assays called for in Tier 1 of the EDSP, in accordance with OMB's recommendation that EPA accept "Other Scientifically Relevant Information" to satisfy the test orders. Additional data indicate Fenbutatin oxide is "very highly toxic" to numerous aquatic organisms (Peck C. et al., 2009). The significant acute and chronic toxicity of fenbutatin oxide at very low doses should be a sufficient basis on which to regulate this chemical, without the need for further testing for endocrine effects in aquatic species.

There is also a compelling body of data on reproductive and developmental toxicity in mammals, including developmental studies in rats and rabbits and multi-generation reproduction studies in rats. EPA considered three such studies in the 2009 Registration Review (Barnes, P. Y. et al., 2009). All three of these studies suggest low potential for endocrine effects and should be considered in lieu of further *in vivo* mammalian testing under the EDSP. In a rat developmental study (Dix, K.M. et al., 1981), mothers exhibited no effects except reduced body weight, and there ***were no developmental effects observed in offspring***. The rabbit developmental study (Dix, K.M.; et al., 1981) considered in the registration review, revealed litter resorptions and post-implantation loss, but these effects were also accompanied by maternal stomach lesions, anorexia,

abortions, and death, which obfuscate interpretation of the data. It should be noted that there were two developmental studies in rabbits (Dix, K.M. et al., 1973a, b) that were not considered by EPA in the 2009 review, which showed no maternal or developmental effects. *In a rat 2 generation reproductive study (Bentley, K. 1990), the only observed parental toxicity was decreased food consumption and body weight at the highest doses tested. There were no effects on fertility, length of gestation, or pup viability, although pup body weight was reduced during lactation.*

III. Summary and Recommendations

Fenbutatin oxide appears to possess low potential for reproductive or developmental toxicity, as observed effects were limited to reductions in body weight. This view is further supported by the fact that several chronic and sub-chronic studies in various mammalian species (rats, rabbits, and dogs) do not provided clear evidence of any specific target organ or toxic effect (Barnes, P. Y. et al. 2009) including reproductive organs and endocrine-mediated effects. Since many of the same apical endpoints which would be addressed by the EDSP Tier 1 *in vivo* assays (body weight, weight and pathology/histopathology of reproductive and other organs) have already been assessed by existing studies, performance of the Tier 1 *in vivo* assays would be duplicative and unnecessary for addressing the relevant endpoints of concern.

If Fenbutatin oxide remains a chemical of concern after reviewing the existing data, then the *in vitro* portion of the Tier 1 battery could be performed to confirm the lack of effects observed in the existing *in vivo* data, prior to any *in vivo* testing. If the *in vitro* data also suggest a lack of endocrine effects, then there is little need for any further testing.

IV. References

- Barnes, P. Y. et al. 2009. Fenbutatin-Oxide (Vendex): Registration Review Scoping Document for Human Health Assessments. U. S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Environmental Fate and Effects Division. Washington, DC 20460.
- Bentley, K. (1990) Reproductive and Fertility Effects with Vendex Technical (IN Y4332-5) Multigeneration Reproduction Study in Rats: Lab Project Number: 128-90: 8388-001. Unpublished study prepared by E. I. du Pont de Nemours and Co./Haskell Laboratory. 1262 p.
- Dix, K.M.; Wilson, A.B.; Stevenson, D.E. (1973a) Toxicity Studies with SD 14114: Teratological Studies in Rabbits Given SD 14114 Orally: Group Research Report TLGR.0052.72. (Unpublished study received May 4, 1976 under 201-367;

- prepared by Shell Research, Ltd., England, submitted by Shell Chemical Co., Washington, D.C.; CDL:224065-A)
- Dix, K.M.; Wilson, A.B.; Stevenson, D.E. (1973b) Toxicity Studies with SD 14114: Teratological Studies in Rabbits Given SD 14114 Orally: Group Research Report TLGR.0052.72. (Unpublished study received Apr 13, 1976 under 201-369; prepared by Shell Research, Ltd., England, submitted by Shell Chemical Co., Washington, D.C.; CDL:097032-A)
- Dix, K.M.; Cassidy, S.L.; Hend, R.W.; et al. (1981) Teratology Study in Rats Given SD 14114 by Gavage: Group Research Report TLGR.80.145.(Unpublished study received Apr 27, 1981 under 201-369; prepared by Shell, Ltd., England, submitted by Shell Chemical Co., Washington, D.C.;CDL:244935-A)
- Dix, K.M.; Cassidy, S.L.; Vilkauls, J.; et al. (1981) Teratology Study in New Zealand White Rabbits Given SD 14114: SBGR.18.055. (Unpublished study received Jul 23, 1981 under 201-369; prepared by Shell Research, Ltd., England, submitted by Shell Chemical Co., Washington, D.C.; CDL:245605-A)
- Peck, C. and Pease, A. 2009. Problem Formulation for Ecological Risk, Environmental Fate, Endangered Species, and Drinking Water Assessments for Fenbutatin-oxide (Vendex). U. S. Environmental Protection Agency. Office of Pesticide Programs, Environmental Fate and Effects Division, Environmental Risk Branch IV. Washington, DC 20460.

Norflurazon, CAS number 27314-13-2
Test order numbers EDSP-105801-19
Test order date: October 29, 2009

I. Introduction

Norflurazon is a selective preemergent herbicide used to control germinating annual grasses and broadleaf weeds (EPA, 1996a). Norflurazon was first registered as a pesticide in the U.S. in 1974, and eight norflurazon products are currently registered. Dietary exposure to norflurazon residues in foods is extremely low and the EPA also expressed minimal concern for risks resulting from occupational exposures.

II. Existing Toxicological Data Related to Endocrine Disruption

In studies using animals, norflurazon generally has been shown to be of low acute toxicity and is practically nontoxic by the oral and dermal routes (EPA, 1996a). With regard to endocrine disrupting potential, a subchronic study in rats evaluated thyroid endpoints, such as those addressed in EDSP Tier 1 pubertal rat tests (Fogleman, R.W. 1971, cited in EPA, 1996b). An increase in thyroid weight of 96% in male rats was observed at the highest dose along with an increase in the incidence of hypertrophic acinar epithelium and colloid depletion of the thyroid. Systemic NOELs were established to be 12.5 mg/kg/day in male rats, and 25 mg/kg/day in female rats. In addition, norflurazon was tested as part of the EPA's ToxCast Phase 1 screen. The NIH Chemical Genomics Center conducted estrogen and androgen receptor binding assays and a Novascreen assay for aromatase inhibition (Akins J, personal communication).

Existing chronic, reproductive and developmental toxicity tests address putative EDSP Tier 2 mammalian endpoints as well. For example, in a 2-year carcinogenicity study, cystic ovaries were observed in female mice at the highest dose level (WARF Institute, Inc. 1975a, cited in EPA, 1996b). No developmental effects were observed in an oral study in rats and the developmental teratogenic NOEL was greater than 400 mg/kg/day. In a study using rabbits, norflurazon caused maternal effects of decreased body weight and clinical toxicity and was fetotoxic at the high dose with dose-related delayed development. No other developmental effect was reported. The maternal NOEL was established to be 30 mg/kg based on decreased weight gain, while the teratogenic NOEL was greater than 60 mg/kg/day (Sandoz Pharmaceutical R&D. 1983, cited in California EPA, 1987). No treatment-related effects were observed at any dose levels in a one-generation reproductive toxicity study in mice (WARF Institute, Inc. 1975c, cited in EPA, 1996b). In a 2-generation reproductive toxicity study in rats, reduced 21-day F2 pup weights and reduced F2 survival were observed that may have been treatment-related. The reproductive NOEL was established to be 150 ppm in this study (Eschbach, B. et al. 1991, cited in California EPA, 2001). Norflurazon is practically nontoxic to avian species on an acute oral and subacute dietary basis but causes reproductive effects in birds at levels as low as 200 ppm (Fink, R. et al., 1980; Fink, R. 1972a & Fink, R. 1972b, cited in EPA, 1996b).

III. Summary and Recommendations

The endocrine disrupting potential of norflurazon has already been addressed in mammalian subchronic, reproductive and developmental studies as well as in avian studies. Since Tier 1 program tests are designed to identify chemicals for further testing in Tier 2, new Tier 1 tests are

unnecessary. Fish reproduction and amphibian metamorphosis studies were not identified. However, information from Tier 1 *in vitro* studies combined with existing mammalian information should be evaluated before conducting any further vertebrate testing.

IV. References

- California Environmental Protection Agency, Department of Pesticide Regulation. 2001. Norflurazon Summary of Toxicological Data. Available at: <http://www.cdpr.ca.gov/docs/toxsums/pdfs/2019.pdf>
- Eschbach, B. et al. 1991. Norflurazon - Two Generation Reproduction Study in Rats. Sandoz Agro Ltd., Basle, Switzerland, report # 91/125.
- Fink, R. 1972a. Final Report: Eight-Day Dietary LC50--Bobwhite Quail: Project No. 620-124. (Unpublished study received on unknown date under 2G1338; prepared by Environmental Sciences Corp.; submitted by Sandoz-Wander, Inc., Homestead, Fla.; CDL: 093577-C)
- Fink, R. 1972b. Final Report: Eight-Day Dietary LC50--Mallard Ducks: Project No. 620-125. (Unpublished study received Nov 14, 1972 under 3G1310; prepared by Environmental Sciences Corp., submitted by Sandoz, Inc., Homestead, Fla.; CDL:092234-G)
- Fink, R., Beavers, J.B., Brown, R. 1980. Final Report: Acute Oral LD50-- Mallard Duck: Project No. 131-113. (Unpublished study received Nov. 3, 1980, under 11273-24; prepared by Wildlife International, Ltd.; submitted by Sandoz, Inc., Crop Protection, San Diego, Calif.; CDL:243646-A)
- Fogleman, R.W. 1971. Evaluation of the Subchronic (90-day) Toxicity Effects of SAN-9789 in Rats: Contract No. 120-546-9-70. Final rept. (Unpublished study received Nov 14, 1972 under 3G1310; prepared by Affiliated Medical Enterprises, Inc., submitted by Sandoz, Inc., Homestead, Fla.; CDL:092233-F)
- Sandoz Pharmaceutical R&D. 1983. Investigation of Teratogenic Potential of Norflurazon in the Rabbit - Segment II. (Sandoz T-1794)
- United States Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances. 1996a. R.E.D. Facts for Norflurazon. Available at: <http://www.epa.gov/REDS/factsheet/0229fact.pdf>.
- United States Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances. 1996b. Norflurazon: Reregistration Eligibility Decision (RED). Available at: www.epa.gov/oppsrrd1/REDS/0229.pdf.
- WARF Institute, Inc. 1975a. Compound 9789: Two Year Feeding Study in Mice. Final Report. (Unpublished study submitted by Sandoz-Wander, Inc.)
- WARF Institute, Incorporated. 1975b. Compound 9789: Reproduction Studies in Rats and Mice: Volume 2: Reproduction Studies in Mice. Final rept. (Unpublished study, including submitter summary, received May 8, 1975 under 5F1602; submitted by Sandoz, Inc., Homestead, Fla.; CDL:094368-A)

Propargite, CAS number 2312-35-8
Test order numbers EDSP-097601-20 - 21
Test order date: October 29, 2009

I. Introduction

Propargite, an organosulphite insecticide, was first registered in the United States in 1969 for control of mites on a variety of field, fruit, and vegetable crops. While propargite is considered a high-use pesticide, with more than 1.3 million pounds applied in California during 2000, its low volatility and high affinity for soil particles restricts its mobility in air and runoff water.¹ As a result, the primary mode of exposure for propargite is through localized spray drift. Studies requested in EPA's 2001 propargite Reregistration Eligibility Decision (RED) concluded that water and residential contexts were not likely modes of exposure.

At the time of the 2001 RED, EPA arranged with the registrant to modify application requirements for propargite and to monitor propargite residue in surrounding water for a period of three years. This monitoring study found no propargite residue in any water sources; this information was submitted to EPA in response to the FR notice announcing the draft list of chemicals for EDSP testing, included in an argument that, based on a lack of residential and water exposure, propargite is ineligible for inclusion among the Phase I chemicals EPA has not responded to this argument, although it was apparently over-ruled by EPA since propargite remains on the Phase I list.^{2,3} As there are no residential uses of propargite, the chronic drinking water level of comparison (DWLOC) is 1400 ppb for the general population, while the estimated chronic environmental concentration (EEC) in surface water is 8.7 ppb and ground water is 0.006 ppb. EPA has therefore stated that it is not concerned with aggregate risks associated with propargite use.⁴

Propargite is classified by EPA as a B2 chemical carcinogen based on the dose-dependent appearance of intestinal tumors observed in a two year cancer bioassay using Sprague Dawley rats, although these findings were not reproduced using Wistar rats and mice. Although EPA's 2001 RED identified no general reproductive or developmental risk in studies using animals, a dietary risk assessment was conducted for the subpopulation of women between 13 and 50 years of age because fetal skeletal malformations were noted in one developmental rat toxicity study.¹⁴ No suitable acute toxicological endpoint was identified among the general population.⁴

The toxicological properties of propargite have been thoroughly examined in several species and have not indicated a need for further endocrine-specific testing. Epidemiological data does not support a developmental impact for this chemical that has been widely used in agricultural applications for more than 40 years.

II. Existing toxicological data

Toxicity information collected for propargite is well-summarized by its assessment in the ToxRef Database.^{5,6} This database, the result of comparative analysis of over 300

multigenerational reproductive and developmental toxicity assays, broadly describes propargite as a chemical whose recognized toxic endpoints tend toward “limited toxicity” with “primarily body weight changes” observed.⁵ One of the first propargite toxicity studies on record, a 1966 two-year dietary exposure using beagles, “failed to reveal any dose-related effects” and identified “[n]o significant microscopic changes in the tissues and organs,” establishing a systemic NOAEL equal to or greater than 900 ppm (or 22.5 mg/kg/day), the highest dose administered.⁷ Similar outcomes were identified in a later study using rabbits, with the dose-dependent pattern of decrease in bodyweight becoming statistically significant once again in the highest dose group, receiving 18 mg/kg/day.⁸ In rats, statistically significant weight change did not emerge among the highest 100 mg/kg/day dose level until after 74 weeks of exposure, establishing an LEL of 100 mg/kg/day and a NOAEL of 45 mg/kg/day.⁹ At doses up to 300 ppm (15 mg/kg/day), weights were comparable between test and control animals in a three generation study using rats, including equivalent mean body weight in pups from birth through maturation.⁹ Additional 90 day dietary exposures using dogs and rats found similar trends in weight loss among animals receiving high doses, although an 18 month carcinogenicity study using albino CD-1 mice at doses up to 1000 ppm (150 mg/kg/day) “revealed no untoward effects toward food consumption, body weights, hematology, and survival.^{10,11,12}” Furthermore, “[v]ariable organ weight changes in the kidney, adrenal, and uterus were not supported by any pathology in these organs.¹²”

While adverse developmental effects of propargite exposure have been noted, existing data from multigenerational reproduction studies have not suggested potential endocrine activity.^{5,6} In its analysis of selected endpoints in reproductive studies using rabbits and rats, EPA’s ToxRef Database found no indications of compound effects on fertility, mating, gestation, embryo implantation, litter size, or pathologies in the testes, prostate, ovaries, uterus, kidneys, liver, adrenal gland, pituitary gland, or thyroid gland.^{5,6} The extent of observed developmental toxicities have related to skeletal abnormalities, with delayed fetal ossification at 6 mg/kg/day in one gestational exposure study using rabbits, resulting in a NOEL for maternal toxicity and fetotoxicity at 2 mg/kg/day.¹³ A similar study using rats identified an increased incidence of missing fetal sternbrae at 25 mg/kg/day, establishing a NOEL and LEL for fetotoxicity at 6 and 25 mg/kg/day, respectively.¹⁴ This is consistent with the results of a three generation study using rats covering a lower dose range, with no developmental or reproductive effects noted up to the highest dose of 15 mg/kg/day (300 ppm).⁷

Ecological toxicity studies have been carried out using fish and birds. Early life studies using fathead and sheepshead minnows suggest that propargite has a relatively low aquatic risk profile, with a NOEC of 16 µg/L and an LC₅₀ below 168 µg/L. Avian reproductive studies using bobwhites and mallard ducks have identified a range of effects. Relatively low exposure to propargite at 84.7 ppm resulted in reduced adult bodyweights, number of eggs laid per female, number of viable embryos, and hatchling survival. At a higher dose of 228 ppm, similar effects were observed, along with a decrease in the number of embryos surviving beyond three weeks, hatching success, hatchling survival and body weight.⁴

Studies of populations exposed to propargite have suggested an elevated proportionate cancer incidence ratio among farm workers exposed to propargite, specifically for gastric cancers of the intestine.¹⁵ While one ecologic study noted increased rates of childhood leukemia in regions where propargite use was highest, no dose-response trends were found.¹⁶

III. Lack of Drinking Water and Residential Exposure

As part of its 2001 reregistration, EPA asked that registrant Chemtura Corporation conduct a three year sampling study to examine the impact on surface and groundwater contamination following institution of wider spray drift buffer zones around areas of propargite product application. This study, which examined community water systems in the four states of highest propargite use, “showed that there was no detection of propargite in any raw water samples throughout the nearly three-year monitoring study. The limit of quantification (LOQ) was considered very low, at 0.025 µg/L (parts per billion).¹⁷” This study also notes that propargite was only identified in two of more than 3,000 groundwater samples in the United States Geological Survey’s National Water Quality Assessment Program, and in none of 382 wells sampled in California (the primary use area of propargite) between 1984 and 1987. Chemtura Corporation noted in the same study that there are no residential uses for propargite registered. While this study (and its early termination due to lack of evidence of propargite water contamination) was requested by EPA, the results of this study were not acknowledged and propargite has remained on the list of Phase 1 EDSP chemicals in spite of the chemical’s failure to meet the exposure requirements for inclusion on this list.

IV. Summary and Recommendations

Existing data used to satisfy EPA’s 2001 RED requirements have yielded valuable information about propargite’s toxicity profile while concurrently providing the information sought in EDSP chemical screening and testing. As studies suggested by EPA have concluded that propargite does not meet the standards for EDSP screening, EPA should provide its rationale for continuing to include this substance on the list of chemicals to be screened in Phase I of EDSP. To justify further testing, EPA should articulate what additional information is required to adequately regulate this substance before additional tests using animals are required.

Although current EDSP criteria state that Phase I chemicals are selected based solely on exposure, EPA has commented that, in the future, it may amend this selection process by considering existing data on known compound effects of chemicals in question². In that context, it is important to note that data from experiments using animals and epidemiological evidence to date have failed to suggest that propargite has the potential to interfere with endocrine processes. ***More specifically, mammalian multigenerational studies have shown no effect on fertility or reproductive endpoints in a variety of species across a broad exposure range.*** More than 800 dogs, rats, mice and rabbits were killed in the studies cited in IRIS alone, and a general consensus from those studies is that propargite is not a priority chemical for EDSP.

We suggest that EPA consider the preponderance of existing animal propargite toxicity data and adopt an integrated approach to further data collection from relevant *in vitro* and epidemiologic sources. Considering past use and historic long-term human exposure to this miticide, we recommend that EPA more closely examine propargite's influence on human physiology by surveying populations exposed during the compound's 41 years of registered use. *In vitro* tests within Tier 1 of EDSP should clearly suggest as-yet undemonstrated endocrine activity before proceeding with further *in vivo* testing. By duplicating existing data, *in vivo* Tier 2 tests would yield no new human-relevant information and would not impact the chemical's regulation. By examining humane approaches before conducting a repetitive, expensive and technically demanding *in vivo* chemical profiling battery, EPA can responsibly generate new data that may be used to gain a broader understanding of propargite's capacity to interact with endocrine-related systems.

IV. References

- ¹ Kegley S et al. Secondhand pesticides: airborne pesticide drift in California. 2003. Docket ID EPA-HQ-OPP-2004-0152-0026.
- ² EPA. Draft List of Initial Pesticide Active Ingredients and Pesticide Inerts to be Considered for Screening under the Federal Food, Drug, and Cosmetic Act. Federal Register 72(116); 33486-33503. June 18, 2007.
- ³ Weiland R. Comment on Proposed Rule: Draft List of Initial Pesticide Active Ingredients and Pesticide Inerts to be Considered for Screening under the Federal Food, Drug, and Cosmetic Act. Docket EPA-HQ-OPPT-2004-0109; FRL-8129-3. Submitted on behalf of Chemtura Corporation. October 29, 2007.
- ⁴ US Environmental Protection Agency. Office of Pesticides and Toxic Substances. 2001. Reregistration Eligibility Decision (RED) for Propargite, Case No. 0243.
- ⁵ Martin et al. Profiling the reproductive toxicity of chemicals from multigenerational studies in the Toxicity Reference Database. *Toxicological Sciences*. 2009; 110 (1); 181-190.
- ⁶ Knudsen et al. Profiling the activity of environmental chemicals in prenatal developmental toxicity studies using the U.S. EPA's ToxRefDB. *Reproductive Toxicology*. 2009; 28; 209-219.
- ⁷ Uniroyal Chemical. 1966. MRID No. 00076402. Unpublished study, EPA IRIS Database.
- ⁸ Uniroyal Chemical. 1982. MRID No. 00105990. Unpublished study. EPA IRIS Database.
- ⁹ Uniroyal Chemical. 1966. MRID No. 00076402. Unpublished study. EPA IRIS Database.
- ¹⁰ Uniroyal Chemical. 1968. MRID No. 00036285. Unpublished study. EPA IRIS Database.
- ¹¹ Uniroyal Chemical. 1964. MRID No. 0007641. Unpublished study. EPA IRIS Database.
- ¹² Uniroyal Chemical. 1979b. MRID No. 00130942. Unpublished study. EPA IRIS Database.

-
- ¹³ Uniroyal Chemical. 1982. MRID No. 00105990. Unpublished study. EPA IRIS Database.
- ¹⁴ Uniroyal Chemical. 1979a. MRID No. 00041386. Unpublished study. EPA IRIS Database.
- ¹⁵ Mills and Yang. Agricultural exposures and gastric cancer risk in Hispanic farm workers in California. *Environmental Research*. 2007; 104; 282-289.
- ¹⁶ Reynolds P et al. Childhood cancer and pesticide use: an ecologic study in California. *Environmental Health Perspectives*. 2002; 110 (3); 319-324.
- ¹⁷ Comment submitted by R. Tim Weiland, Chemtura Corporation, 2007. Docket ID: EPA-HQ-OPPT-2004-0109.

These comments were prepared by:

Catherine Willett, PhD
Science Policy Advisor
People for the Ethical Treatment of Animals

Kristie Sullivan, MPH
Science and Policy Advisor
Physicians Committee for Responsible Medicine

Joseph Manuppello
Research Associate
People for the Ethical Treatment of Animals

Jeff Brown
Research Associate
People for the Ethical Treatment of Animals

Nancy Beck, PhD
Science and Policy Advisor
Physicians Committee for Responsible Medicine