By JRF GLOBAL

JRF SPOTLIGHT: EDSP

March 2015

THE EDSP ABSTRACT CLASSIFIEDS P2 - P3

JRF GLOBAL FACILITATES ED SCREENING FOR YOUR PRODUCTS P4

Around the world

ECPA identifies 66 Products that will need EDSP testing [1] in the EU

The loss of active ingredients under hazard-based criteria for EDs under EU agrochemical Regulation 1107/2009 would likely lead to losses of at least £905 million ($1,416 million) for the UK agriculture alone!
The list is further divided in three scenarios i.e.

01. ED more likely to pose a risk; Human and Ecotox Approvals likely to be lost.

02. ED less likely to pose a risk; Approvals at risk.

03. Potential ED – further information; Required Approvals at risk.

An estimated 17 active substances out of the identified 66, fall under scenario 1.

USEPA releases second list of chemicals for Tier 1 screening

On May 22 2014, the EPA removed hydrazine and hydrochlorofluorocarbon (HCFC-22) from the Second List of Chemicals for Screening. The regulatory body wishes to further analyze occurrence and physicochemical properties of these substances to further determine the suitability of these compounds for Endocrine Disruptor Screening Program (EDSP) testing, in accordance with the Safe Drinking Water Act authority. This follows in the wake of a revised listing of Tier 1 chemicals, first released in 2010.

EDSP testing was introduced by the EPA in 1998 as a two-tiered program to identify potential hormone disruptors using pre-validated assays. Tier 1 testing identifies substances that interact with androgen, estrogen and thyroid hormone systems, while Tier 2 testing calculates dose-dependent adverse effects on the endocrine system.

For more details log on to www.epa.gov

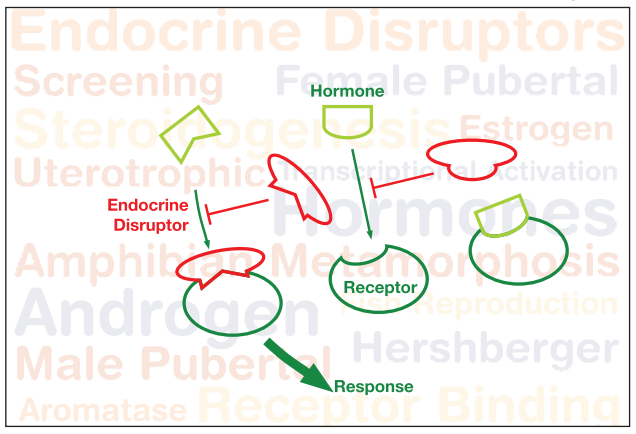
THE ENDOCRINE DISRUPTOR SCREENING PROGRAM

The Endocrine Disruptor Screening Program (EDSP) was established under Section 408(p) of the Federal Food, Drug, and Cosmetic Act (FFDCA), which directed by EPA. Several global labs participated in the development of the assays and the result was the release of ten guidelines by OCSPP in November 2009.

OECD, on the other hand has accepted/released assay guidelines for four out of ten assays mandated by the OCSPP.

The incidences of reduced sperm counts; increased breast cancer incidences; neurological effects like Attention Deficit Disorder; effects in male fish; reptilians (the alligator penis size changes); and many others triggered the testing paradigm. The tests are hence focused on evaluation of the chemicals, which could potentially have an effect on humans and aquatic life, similar to the effect produced by naturally occurring estrogen or similar hormones.

Figure 1



The regulators have offered that the data shall be evaluated on the basis of a "Structured Evaluation Framework" to determine action based on Figure 2.

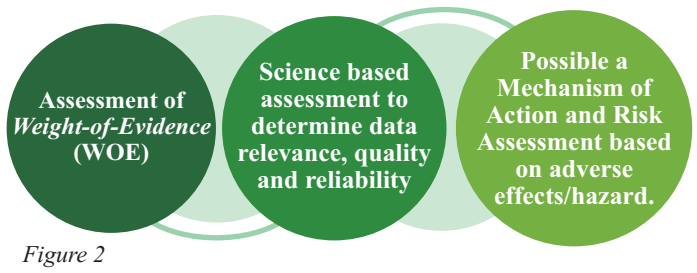


Figure 2

These assays are now being increasingly mandated for the screening of pesticides, commercial and specialty chemicals, and potential environmental contaminants to identify their potential as EDs. The regulators are focusing specifically on estrogen, androgen and thyroid function disruptors, which could potentially be

> ***...The agent or mixture of agents which could potentially interfere or alter the synthesis, secretion, transport, metabolism, binding action, or elimination of hormones that are present in the biological system and could potentially be responsible for altered homeostasis, growth, neurological signaling, reproduction and developmental processes...***

The ultimate purpose of the regulators, is to establish human and environmental relevance of the compounds, which have been proven to have NOEL/NOAEL based on the guideline-driven *in vivo* rodent and non-rodent developmental toxicity tests.

The Principles behind the EDSP Assays

The EDSP assays are primarily focused on genetic expression and receptor binding leading to agonistic/antagonistic effect on the natural expression of the Endocrine systems. The Table - I on the right, should explain the target expression/receptor system being evaluated, which shall be used by the regulators to establish the effects as expressed in Fig. 2.

It can be noted that,

1. An *in vivo* mammalian assay (Uterotrophic & female pubertal rat), *in vivo* Piscean-Reptillian assay (Fish short term reproduction) and *in vitro* assays (ER binding and ER α activation) establish the influence on Estrogen agonistic activities.

2. An *in vivo* female pubertal rat assay & *in vivo* Piscean-Reptillian assay (Fish short term reproduction) and *in vitro* assays (ER binding) help in establishing the indications towards estrogen antagonistic activity potential.

3. An *in vivo* mammalian assay (Hershberger and male pubertal rat), *in vivo* Piscean-Reptillian assay (Fish short term reproduction) and *in vitro* assays (ER binding) identify the influence on Androgen agonistic as well as antagonistic activities.

4. Influence on natural release of Testosterone + Estrogen/Estradiol can be judged on the basis of the *in vivo* male rat pubertal assays + *in vivo* fish short term reproduction as well as an *in vitro* human cell line expressing all the genes in the steroidogenetic pathway.

5. Finally, the hypothalamic pituitary gonadal and/or thyroid effects could be brought to light by the *in vivo* male pubertal, *in vivo* Piscean-Reptillian (amphibian and fish) assays as well as *in vitro* Aromatase assay.

ENDOCRINE DISRUPTION ASSAYS:

The effect on target genes/receptors									
						Steroidigenesis			
Assay	**Test System**		E	AntiE	A	AntiA	T	E	HPG	HPT
***In Vivo* Mammalian assays**									
Uterotrophic	Female rats	✓							
Hershberger	Male and Female Rats				✓	✓			
Pubertal Male	Male rats				✓	✓	✓		✓	✓
Pubertal Female	Female rats	✓	✓						
***In Vivo* Piscean-reptilian assays**									
Amphibian Metamorphosis	Tadpole									✓
Fish Short term reproduction	Fathead minnow	✓	✓	✓	✓	✓	✓	✓	
***In Vitro* Assays**									
ER Binding	Female rat cytosolh	✓	✓						
ER-α Activation	ERα-HeLa-9903	✓							
AR Binding	Male rat cytosol			✓	✓				
Steroidogenesis	H295R					✓	✓		
Recombinant Aromatase	Cyp19							✓	

Legend

A	Androgen
AR	Androgen Receptor
E	Estrogen/Estradiol
ER	Estrogen Receptor
HPG	Hypothalamic Pitutary Gonadal
HPT	Hypothalamic Pitutary Thyroid
T	Testosterone
H295R	H295R Human Adreno-carcinoma Cell Line
Cyp-19	Microsomes isolated from the cell lines expressing recombinant human CYP19 and cytochrome P450 reductase.
Tadpole	*Xenopuslaevis* Tadpole
Fathead minnow	Fathead minnow (*Pimephalespromelas*)

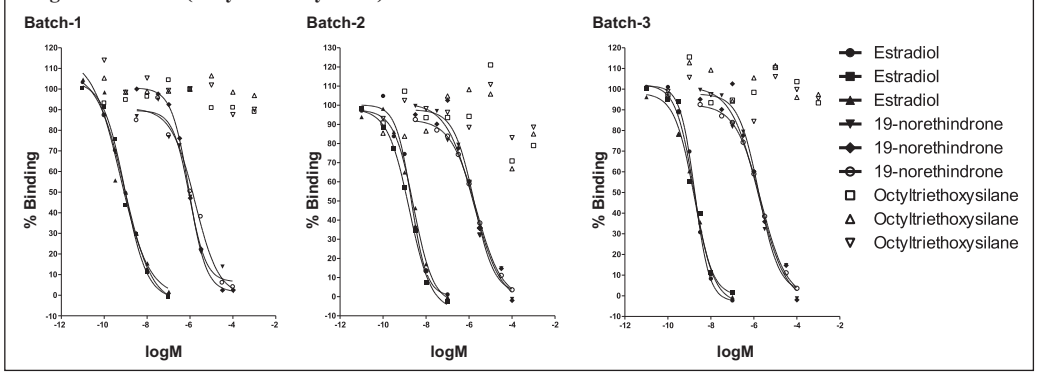
Regulatory evaluation will be based on the interpretation of the matrix data of the results obtained from the above parameters.

VALIDATION OF ENDOCRINE DISRUPTOR SCREENING TEST BASED ON ESTROGEN RECEPTOR BINDING ASSAY USING RAT UTERINE CYTOSOL

Pandey S. P., Karurkar A. P., Gaikwad S. G., Patel M. V. and Nagane R.M.

Environmental chemicals that compete with endogenous estrogens have the potential to interact with estrogen receptor (ER) and interfere with normal estrogen activity, resulting in endocrine disruption. Thus, interactions of the test items with ER constitute a direct, simple evaluation of its estrogenic potential in vertebrate species. The objective of this study was to demonstrate the inhibition of estrogen receptor binding of[3H]-17β-Estradiol in rat uterine cytosol by a competitor chemical, thus validating ER binding as a potential tool for screening environmental chemicals under laboratory conditions at JRF. Cytosols were prepared at JRF from ovariectomized Sprague Dawley rats. Three independent runs of saturation binding experiments were performed to characterize the cytosol preparation and to ensure that the ER activity is sufficient for the competitive assay. Three independent runs of competitive binding assay were performed with 17ß-Estradiol, 19-norethindrone and octyltriethoxysilane to validate the assay in compliance with EPA guidelines classification. Assay reaction mixture consisted of cytosol, master mixture (TEDG + PMSF assay buffer + [3H]-17β-estradiol) and competitor chemical. The inhibitory effect of the competitor on ER binding of [3H]-17β-estradiol was evaluated by measuring the amount of bound [3H]-17β-estradiol by liquid scintillation counter. The cytosols assayed showed a plateau of maximum specific binding and a linear Scatchard plot indicative of one-site binding. The competitive binding assay parameters were in compliance with EPA guideline OPPTS 890.1250 (17ß-Estradiol and 19-Norethinidrone-interactive and octyltriethoxysilane non-interactive, with ER). The validation study generated reproducible data during all the runs of saturation as well as competitive binding assays in compliance with EPA guidelines establishing the credentials of JRF as a capable lab in conduct of the saturation and competitive binding experiments.

Competitive Binding Reference Substance (Estradiol), Weak Positive Control (19-Norethindrone) and Negative Control (Octyltriethoxysilane)

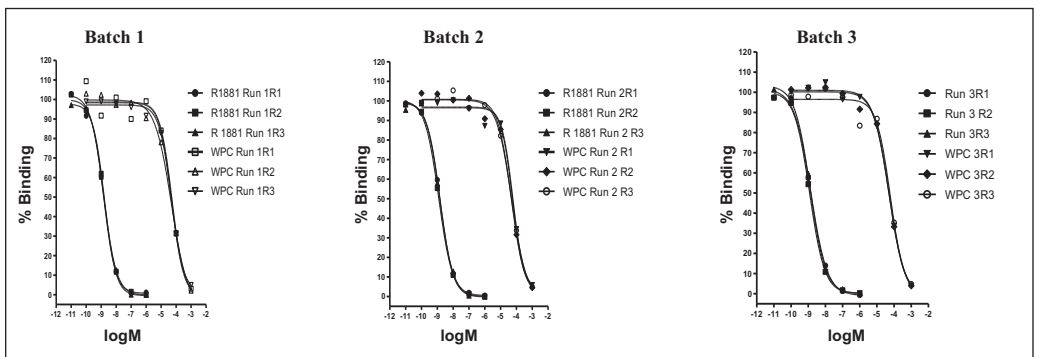


VALIDATION OF ENDOCRINE DISRUPTOR SCREENING TEST BASED ON ANDROGEN RECEPTOR BINDING ASSAY USING RAT PROSTATE CYTOSOL

Pandey S. P., Karurkar A. P., Patel M. V. and Nagane R.M.

The Androgen Receptor (AR) plays a pivotal role in the development and maintenance of the male and female reproductive systems. Various chemicals can potentially interfere with normal androgen activity by binding with androgen receptors, resulting in endocrine disruption. The objective of this study was to demonstrate the inhibition of AR binding of R1881 in rat ventral prostate cytosol by weak positive control (Dexamethasone). Rat prostate cytosol was prepared at JRF from castrated Sprague Dawley rats. Three independent runs of saturation binding experiments were performed to characterize the cytosol preparation and ensure the AR activity is sufficient for the competitive assay. Three independent runs of competitive binding assays were performed with known reference substance (R1881) along with dexamethasone. Assay reaction mixture consisted of cytosols, substrate, and test or control substance in a common reaction tube.

The inhibitory effect of the dexamethasone on androgen receptor binding of R1881 was evaluated by measuring the amount of bound 3H-R1881 by liquid scintillation counter. The saturation binding parameters met the acceptance criteria. The cytosol assayed showed a plateau of maximum specific binding and a linear Scatchard plot indicative of one-site binding. The competitive binding assay parameters were in compliance with EPA guideline, OPPTS 890.1150. The average curve across runs crosses 50% for dexamethasone, therefore it was classified as androgen receptor binder. The data revealed that the cytosols had sufficient receptors and specific binding was at one site (saturation binding). It was also established that the cytosols were also capable of competitive binding. The results with the reference substance, weak positive control met the EPA acceptance criteria. This establishes the capability of JRF in preparation of the rat prostate cytosols, conduct of saturation binding and competitive binding assays.



Competitive Binding R1881 and Weak Positive Control Run

VALIDATION OF ENDOCRINE DISRUPTOR SCREENING TEST BASED ON AROMATASE ASSAY (HUMAN RECOMBINANT)

Karurkar A. P., Pandey S. P., Rana J.R., Patel M. V. and Nagane R.M.

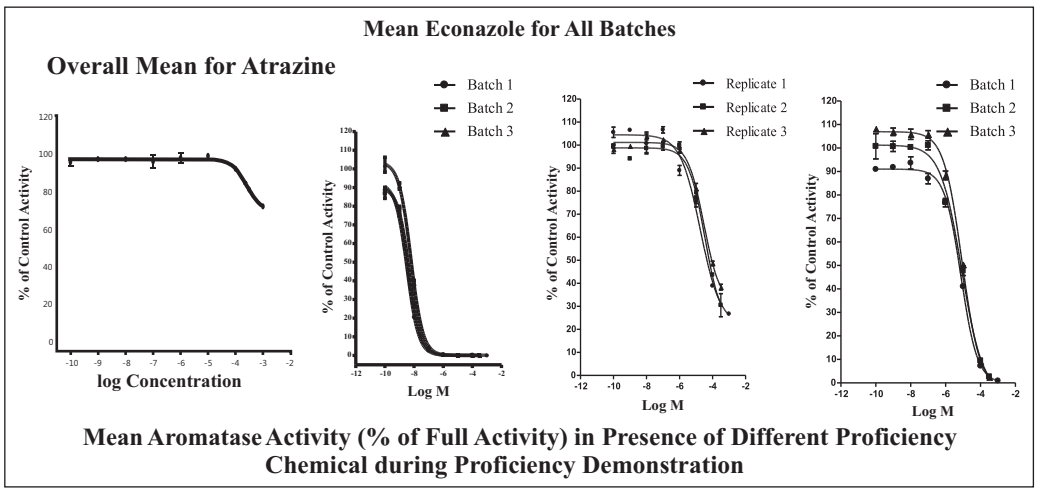
Aromatase is a cytochrome P450 enzyme complex, which converts androgens to estrogens during steroidogenesis. Various chemicals could potentially inhibit/alter the aromatase levels resulting in altered levels of estrogen, resulting in endocrine disruption. Estradiol, the most potent endogenous estrogen in humans, is biosynthesized from androgens by the cytochrome P450 enzyme complex called aromatase. Present study was performed to validate the Aromatase (Human Recombinant) assay at JRF. 4-Hydroxyandrostenedione (4-OH ASDN) was compared as positive control against atrazine, econazole, nitrofen and fenarimol as proficiency chemicals. Three independent experimental runs were performed with positive control followed by 3 independent runs with the above proficiency chemicals. Assay reaction mixture consists of microsomes, substrate, cofactors and test chemicals under controlled conditions for the maximal enzyme activity.

[3H]- and rostenedione was used along with the non-radiolabeled ASDN as substrate. Aromatase converts androstenedione (ASDN) into estrone. The formation of $3H_2O$ was measured by liquid scintillation counter. Data was interpreted as aromatase activity (nmol/mg-protein/min.) and % of full Activity. The aromatase activity was > 0.15 nM/mg-protein/min. during all the validation runs. Overall response curve parameters for 4-OH ASDN concentrations in these experiments were analysed by Prism software.

The results were fully within the acceptance criteria prescribed as per the EPA guideline OPPTS 890.1200. 4-OH-ASDN at concentrations ranging from 0.1-10,000 nM resulted in a sigmoidal dose response curve ranging from no inhibition to almost full inhibition. The experimental data of proficiency chemicals complied in the EPA classification category (atrazine- non-inhibitor, econazole – strong inhibitor, fenarimol and nitrofen- inhibitor). The results established their reproducibility during three independent runs of the positive control (4-OH ASDN) with proficiency chemicals.

The responses in all the three tests were reproducible and met the acceptance criteria defined by EPA guidelines.

Mean Econazole for All Batches



Mean Aromatase Activity (% of Full Activity) in Presence of Different Proficiency Chemical during Proficiency Demonstration

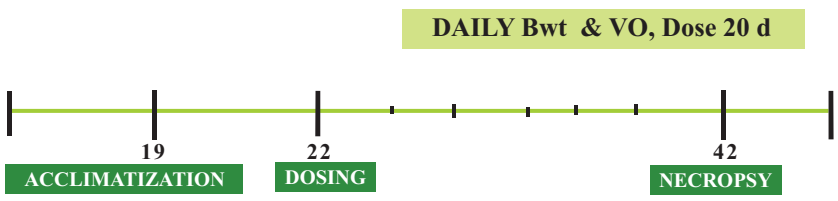
EFFECTS OF 1-CHLORO-2-NITROBENZENE AND METHOXYCHLOR ON INTACT JUVENILE/PERIPUBERTAL FEMALE RATS

Patel M.V., Piccirillo V., Paneliya S.M., Posia M.P., Brahmankar M.G. and Lonkar P.S.

Female Pubertal Assay, was validated as a part of the Tier I battery in Endocrine Disruptor Screening Program (EDSP) in compliance with OPPTS 890.1450. The positive controls used were; 1-Chloro 2-Nitrobenzene (CNB; doses 25 and 100 mg/kg) and Methoxychlor (MTH; doses 12.5 and 50 mg/kg). The control groups were dosed with corn oil. Sixteen F1 female rats were used per dose group.The rats were dosed by oral gavage once a day from PND 22 to 42. No clinical signs were observed in rats treated with corn oil and MTH. Salivation was seen in the rats treated with CNB (25 and 100 mg/kg). No changes in body weight and body weight gain were observed in rats treated with CNB. A significant decrease in final body weight and body weight gain was observed in rats treated with MTH. No treatment related difference was observed in feed consumption in rats treated with CNB and MTH. Statistical significant delay in mean age at incomplete vaginal opening was observed in rats treated with CNB.

The mean age at first estrus was delayed (not statistically) in MTH treated groups when compared with the control group. Significant decrease in percent regular cycling was observed in CNB treated dose groups. Cholesterol and ALT, and total bilirubin were significantly increased at 25 and 100 mg CNB/kg dose groups, respectively. Significant changes were observed in liver, kidney and ovary weights in the rats treated with CNB. Splenomegaly was observed in rats treated with 100 mg/kg CNB which was microscopically diagnosed as sinusoidal congestion and erythroid cell hyperplasia. Positive endocrine disruptor effects such as delay in pubertal development, reduction in sex organ weight and decrease in T4 levels were observed at the highest dose tested of CNB; and reduction in body weight, decrease in T4 levels were observed at the highest dose tested of MTH. Thus, the methodology and results obtained from the present study establishes achievement of the performance criteria as per the U. S. EPA OPPTS 890.1450 guideline.

FEMALE PUBERTAL ASSAY



END POINTS
Vaginal Opening, Estrous cycle, organ Wt., and Histopatholgy (Uterus, Ovaries & Thyroid)

Dosing: Orally daily at same time. Necropsy after 2 hr of last dose

HERSHBERGER BIOASSAY (ANDROGEN AGONIST) OF TESTOSTERONE PROPIONATE IN SURGICALLY CASTRATED PERIPUBERTAL MALE RATS

Patel M.V., Piccirillo V., Paneliya S.M., Posia M.P., Brahmankar M.G. and Lonkar P.S.

The current study presents the effects of Testosterone Propionate (TP), as Androgen Agonist, employing the Hershberger Bioassay, using castrated male rats (Wistar, RccHan: WIST). The study was undertaken in compliance with OECD N° 441 and the U.S. EPA OPPTS 890.1400 guidelines. TP was administered daily by subcutaneous injection in three treatment groups (0.2 (G2), 0.4 (G3) and 0.8 (G4) mg/kg b. wt.) in male rats for a period of ten consecutive days at approximately 24 hour intervals. Vehicle control group (G1) received distilled water. No treatment related mortality and clinical symptoms were observed during the study. No treatment related significant difference were observed in the mean body weight and feed consumption of rats in all treatment groups.

However, the body weight gain was significantly increased in the G2 to G4 dose groups. Significant increase in terminal body weight was observed in G3 and G4 dose groups after log transformation. Significant increases in absolute and log transformed weight of all five androgen dependent tissue were observed in G2, G3 and G4. Significant increases in kidney absolute and log transformed weight were observed in G3 and G4 dose groups and significant increase in liver log transformed weight was observed in G3 dose group. Grossly, dose dependent increase in size of ventral prostate, seminal vesicles plus coagulating glands, levator ani and bulbocavernous muscles, and Cowper's glands were observed in all the dose groups and in glans penis only in G4 group as compared to G1 group. The results of the present study indicate that Testosterone Propionate is an androgen agonist in castrated male rats. Thus the methodology and result of the present study, achieve the performance criteria for androgen- dependent organ weight as per the OECD 441 and U. S. EPA OPPTS 890. 1400 guidelines.

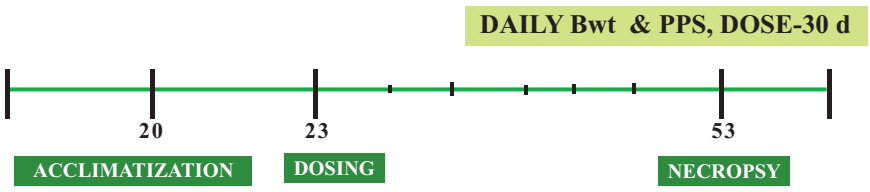
EFFECTS OF 1-CHLORO-2-NITROBENZENE AND DIBUTYL PHTHALATE ON INTACT JUVENILE/PERIPUBERTAL MALE RATS

Patel M.V., Piccirillo V., Paneliya S.M., Posia M.P., Brahmankar M.G. and Lonkar P.S.

Male Pubertal Assay, was validated in the Tier I battery under EDSP, using positive control substances, as per U.S. EPA OPPTS 890.1500. 1-Chloro 2-Nitrobenzene (CNB: 25 and 100 mg/kg b.wt/day) and Dibutyl Phthalate (DBP: 500 and 1000 mg/kg b.wt/day) were used for validating the procedure with one control group. Sixteen F1 male rats per dose group were used and same were dosed once daily from PND 23 to 53. No clinical signs were observed in rats treated with corn oil and 25 mg/kg CNB. However salivation was seen in rats treated with CNB (100 mg/kg b.wt/day) and DBP (500 and 1000 mg/kg b.wt/day). Delay in separation was seen in male rats from all treated groups. Androgen dependant sex organ was significantly decreased in the 100 mg/kg b.wt/day CNB group and at 500 and 1000 mg/kg b.wt/ day DBP dose groups.

A significant increase in liver and adrenal (relative weight) weights were observed in groups treated with CNB and increase in liver weight was also noted for the 1000 mg/kg b.wt/day DBP. Grossly, reduction in size of testes (at 25 mg/kg b.wt/day CNB and 500 and 1000 mg/kg b.wt/day DBP) and splenomegaly (at both the CNB groups) were observed. Microscopic changes in spleen (at 100 mg/kg b.wt/day CNB) and testes (in both DBP groups) were observed. Significantly decreased T4 (log transformed) was seen in the 100 mg/kg b.wt/day CNB group as well as both the 500 and 1000 mg/kg b.wt/day DBP groups as compared to the vehicle control group. Significantly decreased serum total testosterone level was observed at 500 mg/kg b.wt/day of DBP as compared to the vehicle control group. Positive endocrine disruptor effects (delay in pubertal development, reduction in androgen dependent sex organ weight, microscopic changes in spleen and testes, decrease in T4 level) were observed at the highest dose level tested of CNB and DBP. Thus the methodology and results obtained from the present study, establishes achievement of the performance criteria as per U. S. EPA OPPTS 890.1500 guidelines.

MALE PUBERTAL ASSAY



END POINTS
Preputial seperation, Organ wt.(SVGC, VP, LABC, Testis, Epididymis, Pituitary, Adrenal, Thyroid & Kidney), Histopathology Testis, Epididymis

Dosing: Orally daily at same time. Necropsy after 2 hr of last dose

HERSHBERGER BIOASSAY (ANDROGEN ANTAGONIST) OF FLUTAMIDE CO-ADMINISTERED WITH TESTOSTERONE PROPIONATE IN SURGICALLY CASTRATED PERIPUBERTAL MALE RATS

Patel M.V., Piccirillo V., Paneliya S.M., Posia M.P., Brahmankar M.G. and Lonkar P.S.

Effects of the Flutamide (FLU), co-administered with the reference Testosterone Propionate (TP) as Androgen Antagonist were quantified in the Hershberger Bioassay using castrated male rats (Wistar, RccHan: WIST). The study was undertaken as per OECD N° 441 and the U.S. EPA OPPTS 890.1400. The Flutamide was administered daily by oral gavage in three treatment groups (0.3 (G2), 1 (G3) and 3 (G4) mg/kg b.wt./day) for a period of ten consecutive days in concert with daily reference Testosterone Propionate (0.4 mg/kg b.wt./day) by subcutaneous injection. The negative control group rats were administered 0.4 mg/kg b.wt./day TP only. No treatment related mortality or clinical symptoms were observed during the study. No treatment related significant difference was observed in the mean body weight of rats in all the treatment groups as compared to the negative control group in both the trials.

However, statistically significant reduction in the body weight gain and feed consumption was observed in G3 and G4 dose groups as compared to the G1 group. Terminal sacrifice body weights of the rats from the various treatment groups were comparable with the negative control group. The absolute weight of ventral prostate, levator ani and bulbocavernous muscles, seminal vesicles plus coagulating glands, cowper's glands and glans penis were significantly decreased in all Flutamide treated dose groups as compared to the negative control group. No treatment related pathological changes were observed during study. The results of the present study indicate that Flutamide (FLU) is androgen antagonist (anti-androgenic) in castrated male rats at the dose level of 0.3 mg/kg b. wt./day and greater when co-administered with Testosterone Propionate (0.4 mg/kg b. wt./day). Thus the methodology and result of the present study achieve the performance criteria for androgen- dependent organ weight as per the OECD 441 and U. S. EPA OPPTS 890. 1400.

UTEROTROPHIC BIOASSAY OF 17 α -ETHYNYLESTRADIOL IN OVARIECTOMIZED ADULT FEMALE RATS

Patel M.V., Piccirillo V., Paneliya S.M., Posia M.P., Brahmankar M.G. and Lonkar P.S.

Effects of the 17α-ethynylestradiol (EE), as estrogen agonists, in the Uterotrophic Bioassay, were quantified using ovariectomized (OVX) female rats (Wistar, RccHan: WIST) as per the guideline of OECD N° 440 and OPPTS 890.1600. The ovariectomized (OVX) female rats were given 17α- ethynylestradiol by subcutaneous injection at the dose level of 0.3 (G2), 1 (G3), 3 (G4) and 10 (G5) µg/kg b. wt. daily for a period of three consecutive days at approximately 24 hour intervals.

The vehicle control (G1) group rats were administered corn oil. No treatment related mortality, clinical symptoms or variations in mean body weight were observed up to the dose level of 10 µg/kg b. wt. dose group.

However, significantly low body weight gains were recorded in G3 to G5 (1, 3 and 10 µg/kg b. wt.) dose groups, while feed consumption of was significantly low only in 10 µg/kg b. wt. dose group as compared to the G1 group. The absolute and relative wet and blotted uterus weight and those in log transformation were significantly increased in all the dose groups (except absolute blotted uterus weight in G2 group) as compared to the control group. Pathological examination did not show any lesion in any of the groups. The results indicated that 17α-ethynylestradiol is estrogen agonist in ovariectomized (OVX) female rats at dose level of 0.30 µg/Kg b.wt./day and greater. The methodology and result of the present study achieve the slightly higher acceptance criteria at 0.06% (instead of 0.04%) for blotted vehicle control uterine weight as per the OECD 440 and U. S. EPA OPPTS 890. 1600 guidelines.

Customers are not dependent on us,
We are dependent on them.
They are not outsiders in our business,
They are a part of it.

- *Mahatma Gandhi*

References:

1. UK Agriculture and Horticulture Development Board (AHDB) - EU Market Report, December 9th, 2014 published on December 11, 2014)
2. Terry F. Quill presentation (Quill Law Group LLC Washington, DC 20006) Gradient Breakfast Seminar May 11,2010

JRF GLOBAL facilitates Endocrine Disruptor Screening for your products

JRF Global is proud to be the first laboratory in Asia to take a lead in validating the EDSP assays in compliance with the OCSPP as well as OECD guidelines! We are all geared-up to offer assays included in the Tier 1 Screening Battery, on the basis of the latest revalidation in compliance with these guidelines.

JRF has a strong and proven capability to;

- Design the experiment programs for assessing possible endocrine disruptive effects as per the EPA and OECD guidelines.
- Offer multiple products in parallel for the EDSP studies, using state-of-the-art equipment.
- Excel in ensuring delivering the reports by our dedicated team of experts, within the committed timelines, ensuring rapid turnaround for the benefit of our revered sponsors.
- Strong and professional project management team.
- Tailor-made guidance / consultancy and testing services for EDSP by combining excellent project communication and support from our dedicated team of experts.
- With rapid turnaround times, we will attempt to help you in shortening timelines with our testing program.

Our promising timelines for Tier 1 Screening Battery

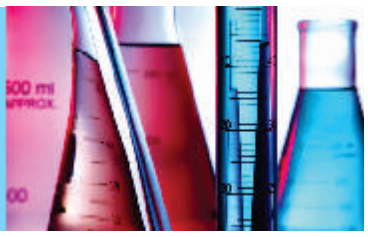
***In Vitro* Assays**			
Assays	**Guidelines/References**	**Endpoints**	**Study Duration**
Androgen Receptor Binding	OPPTS 890.1150	Saturation Binding- Estimation of Kd and Bmax, Competitive Binding with methyl trienolone, - IC50, Receptor Binding Assay(RBA)	Report: 30 days
Aromatase	OPPTS 890.1200	Measurement of generated Estrone by Liquid scintillation counting, Ic50	Report: 30 days
Estrogen Receptor Binding	OPPTS 890.1250	Saturation Binding- Estimation of ofKd and Bmax, Competitive Binding - 3 Consecutive runs, IC50, Receptor Binding AssayRBA	Report: 30 days
Estrogen Receptor Transcriptional Activation	OPPTS 890.1300	Quantitative measurement of bioluminescence product (Luminescence signal) by Luminometry	Report: 30 days
Steroidogenesis	OPPTS 890.1550	Measurement of the levels of Testosterone and 17β-Estradiol produced by cell line with LC-MS/MS, 17β-Estradiol will be measured after Derivatization with Dansyl Chloride, Cell Viabilityat end of treatment using Invitrogen's LIVE/DEAD Viability kit	Report: 30 days

***In Vivo* Assays**			
Assays	**Guidelines/References**	**Endpoints**	**Study Duration**
Uterotrophic Assay	OPPTS 890.1600	**Organ Weight:** Uterine Weight both "Wet" Weight and "Dry" Weight **Optional Histopathology:** Uterus	18 Weeks
Hershberger Bioassay	OPPTS 890.1400	**Organ Weight:** Ventral Prostate, Seminal Vesicles, LevatorBulbocavernosus Muscle, Cowpers Glands, Paired Epididymides, Paired Testis Weight, Glans Penis Optional Organ Weight: Liver, Kidneys, Adrenals **Optional Hormone Analysis:** T3, T4, Testosterone, LH	18 Weeks
Pubertal Development And Thyroid Function In Intact Juvenile/Peripubertal Male Rats	OPPTS 890.1500	**Sexual Maturation:** Daily Assessment of Preputial Separation **Blood Chemistry** **Organ Weights:** Seminal Vesicles/Coagulating Glands, Ventral Prostate, Dorsal Prostate LevatorAni Plus Bulbocavernosus Muscle, Epididymis, Testis, Thyroid (Post Fixation), Liver, Kidney, Adrenals, Pituitary **Histopathology:** Epididymis, Testis, Thyroid, Kidney **Hormone Analysis:** T3, T4, THS, Testosterone	24 Weeks
Pubertal Development And Thyroid Function In Intact Juvenile/Peripubertal Female Rats	OPPTS 890.1450	**Sexual Maturation:** Daily Assessment of Vaginal Separation **Blood Chemistry** **Organ Weights:** Uterus (Blotted), Ovaries, Thyroid (Post Fixation), Liver, Kidney, Pituitary, Adrenals **Histopathology:** Uterus, Ovary, Thyroid, Kidney **Hormone Analysis:** T3, T4, THS	24 Weeks

Crop Protection Sector

Pharmaceutical Sector

Speciality Chemicals Sector



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