ANNEX 5-1 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent
Tributyltin chloride

(1) ***in vivo*** **Tests**

Competent authorities	Dose Assay and Test	μg/kg/day				mg/kg/day			Comment
		10	30	100	300	2	20	200	
		0.15	0.45	1.5	4.5	30	300	3,000	ppm
MoE	One-Generation Study	D	D	C F1 males: Low values of spleen (absolute/relative) weights	C F0 dams: High values of food consumption F1 males: Low values of spleen (absolute/relative) weights P F1 males: High values of percent neutrophils	A*			Diet 42 days
METI	Uterotrophic Assay					–	–	–	Subcutaneous 3 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related (suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).
Results of METI(–:negative, +:positive)
<Findings observed at A*>
*F0 dams: Low values of body weight, weights of thymus (absolute/relative), spleen (absolute/relative), ovary (absolute/relative) and uterus (absolute/relative).
*F1 males: Low values of spleen (absolute/relative) weight.
*F1 females: Low values of body weight and thymus (absolute/relative) weight.
(The underlined findings are those reported in certain animals.)

(2) ***in vitro*** **Assay**

Competent authorities	Assay	Results	Range	Comment
MoE	ERα binding assay	$IC_{50}=4.0\times10^{-6}$M	10^{-9}–10^{-5}M	difficult to distinguish from cell toxicity
	ERβ binding assay	$IC_{50}=6.3\times10^{-6}$M	10^{-9}–2.2×10^{-5}M	difficult to distinguish from cell toxicity
	E-screen	8% of E2 at 10^{-12}M	10^{-14}–10^{-4}M	cell toxicity at 10^{-9}–10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-8}–10^{-5}M	cell toxicity at 10^{-6}–10^{-5}M
	AR reporter gene assay(antagonist)	–	10^{-8}–10^{-5}M	cell toxicity at 10^{-6}–10^{-5}M
	AR binding assay	$IC_{50}=4.1\times10^{-6}$M	10^{-6}–10^{-3}M	difficult to distinguish from cell toxicity
	TRα	–	$<2\times10^{-5}$M	
	TRβ	–	$<2\times10^{-5}$M	

IC_{50} (the concentration that inhibits 50% combination of labeled hormone and receptor), PC_{50} (the concentration that induces 50% activity of chemiluminescent intensity induced by E2, etc.), Ecx_{10} (the concentration that shows chemiluminescent intensity of 10 times as much as the background value) obtained were recorded. When these values were not obtained, the maximum reaction value and the concentration were recorded.
Values were analyzed for statistically significant differences, and –indicates no activity.

ANNEX 5-2 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Di-n-butyl phthalate

(1) ***in vivo*** **Tests**

competent authorities	Dose Assay and Test	μg/kg/day					mg/kg/day				Comment
		31	63	125	250	500	40	200	250	1,000	
MoE	One-Generation Study	S F1 male: Increase of dorsal prostate (abs. and relatative weight), Delay of preputial separation, Decrease of mRNA (prpstate; ERα). F1 female: Advance of vaginal opening	S F1 male: Decrease of pituitary (ab. and relative weight), Increase of pdorsal prostate (abs. and relative weight)	S F1 female: Decrease of thyroid (absolute and relative weight)	S F1 male: Increase of dorsal prostate, thyroid, epidid. and penis(abs. and relative weight) F1 female: Decrease of thyroid(abs. and relative weight)	S F1 male: Decrease of pituitary (abs. and relative weight), Decrease of mRNA (prpstate; ERα). F1 female: Advance of vaginal opening			A		l42-day oral dosing
METI	Uterotrophic Assay						–	–		–	Subcutaneous 3 days
	Hershberger Assay										Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI(–: negative, + : positive)
A*
* F1 pups: Depression of day 0 viability.
* F1 male: Decrease of AGD(absolute and relative), and mRNA(prpstate;ERα) concentration. Defect, atrophy and hypoplasia of genital organs and accessory reproductive organs
* F1 female:Decrease of body weight, pituitary and thyroid weights (absolute and relative), mRNA(Uterus; ERα) concentrations. Decrease of No. of live pups and advance of vaginal opening.

ANNEX 5-2 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent (continued)

Di-n-butyl phthalate

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range
MoE	ERα binding assay	22% at 10^{-4}M	10^{-9} - 10^{-4}M
	ERβ binding assay	7% at 10^{-4}M	10^{-9} - 10^{-4}M
	E-screen	–	10^{-9} - 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} - 10^{-5}M
	AR reporter gene assay(antagonist)	–	10^{-6} - 10^{-5}M
	AR binding assay	17% at 10^{-4}M	10^{-6} - 10^{-3}M
	TRα	–	<2 x 10^{-5}M
	TRβ	–	<2 x 10^{-5}M

– indicates no activity

ANNEX 5-3 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Octachlorostyrene

(1) ***in vivo*** **Tests**

competent authorities	Dose / Assay and Test	μg/kg/day				mg/kg/day				Comment
		2.4	12	60	300	2	20	50	200	
MoE	One-Generation Study	C*	D	C* P*	C* P*			A*		Oral 42 days
METI	Uterotrophic Assay					–	–		–	subcutaneous,Rdays
	Hershberber Assay									Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI (–: negative , + : positive)

‹ Signs of A*›
* F_0 dams : hypertrophy of the centrilobular hepatocyte, slight proliferation of mammary gland lobule • Adecrease in food consumption and number of implantations.
* F_1 newborns : decrease in viability index(all newborns died from day 1 to day 9 of lactation).
* F_1 offspring : decrease in body weight.
(The underline showed the view that have already been reported.)

‹ Signs of C*›
*F_1 offspring : increase in absolute and relative epididymides weights in the 300μg/kg.
*F_1 offspring : increase in corpora lutea in the 2.4μg/kg and decrease in absolute and relative uterus weights in the 60μg/kg.

‹ Signs of P*›
*F_1 offspring : increase in mRNA expression in ERβ of the uterus in the 60 and 300μg/kg .

ANNEX 5-3 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent(continued)

Octachlorostyrene

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range	Comment
MoE	ERα binding assay	20% at 10^{-4}M	10^{-11} – 10^{-4}M	
	ERβ binding assay	19% at 10^{-5}M	10^{-11} – 2.2 x 10^{-5}M	
	E-screen	22% of E2 at 10^{-5}M	10^{-10} – 10^{-5}M	
	AR reporter gene assay (agonist)	13% at 10^{-6}M	10^{-6} – 10^{-4}M	cell toxicity at 10^{-5} – 10^{-4}M
	AR reporter gene assay (antagonist)	35% at 2 x 10^{-5}M	10^{-6} – 10^{-4}M	cell toxicity at 3 x 10^{-5} – 10^{-4}M
	AR binding assay	21% at 10^{-4}M	10^{-6} – 10^{-3}M	
	TRα	–	<2 x 10^{-5}M	
	TRβ	–	<2 x 10^{-5}M	

– : indicates no activity

ANNEX 5-4 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Benzophenone

(1) ***in vivo*** **Tests**

competent authorities	Dose Assay and Test	μg/kg/day			mg/kg/day									Comment
		2	10	50	1	2	5	10	20	50	100	200	500	
MoE	One-Generation Study	C F0 Dam: Increase of food intakes.	C F0 Dam: Increase of food intakes. P F1 male: Increase of LH conc. in serum	C F1 male: Decrease of dorsal prostate, (absolute and relative weight) P F1 male: lower of spleen (absolute and relative weight) F1 female: Decrease of estradiol conc. in serum					A		A			Oral42days
METI	Uterotrophic Assay estrogenic						–			–			+	subcutaneous,7days ovariectomized
	Uterotrophic Assay antiestrogenic						–			+			+	subcutaneous,7days ovariectomized
	Uterotrophic Assay					–			–			–		subcutaneous,3days
	Hershberger Assay androgenic				–			–			–			Oral10days, gonadectomized
	Hershberger Assay antiandrogenic				–			–			–			Oral10days, gonadectomized
	Two-Generation Study													Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported.
However, their biological/toxicological significance remains to be elucidated at present(pending).

Results of METI (– :negative, +:positive)

A*1

* F0 Dam: Increase of water intakes
* F1 Male: Increase of kidneys weight (absolute and relative weight), increase of LH concentration, decrease of estradiol and FSH concentrations in serum.
* F1 Female: Increase of adrenals weight (absolute and relative weight), increase of LH and decrease of estradiol concentrations in serum.

A*2

* F0 Dam: Increase of food intakes, decrease of No. of live pups at day 0, extension of gestation period.
* F1 Pups: Decrease of No. of live pups and viability at day 0.
* F1 Male: Increase of AGD(absolute and relative), higher of LH in serum, decrease of residual nipples and FSH concentration in serum, degeneration of seminiferous tubule.
* F1 Female: Increase of kidneys weight (absolute and relative weight), and LH concentration in serum.

ANNEX 5-4 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent(continued)

Benzophenone

(2) ***in vitro*** **Assay**

competent authorities	**Assay**	**Results**	**Range**
MoE	ERα binding assay	5% at 10^{-4}M	10^{-9} – 10^{-4}M
	ERβ binding assay	5% at 10^{-5}M	10^{-9} – 10^{-4}M
	E-screen	PC_{50}=3.7 x 10^{-5}M	10^{-9} – 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} – 10^{-4}M
	AR reporter gene assay(antagonist)	IC_{50}=1.1 x 10^{-4}M	10^{-6} – 10^{-3}M
	AR binding assay	IC_{50}=4.9 x 10^{-4}M	10^{-6} – 10^{-3}M
	TRα	–	<10^{-4}M
	TRβ	–	<10^{-4}M

– : indicates no activity

ANNEX 5-5 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Di-cyclohexyl phthalate

(1) ***in vivo*** **Tests**

competent authorities	Dose Assay and Test	μg/kg/day				mg/kg/day							Comment
		1.6	8	40	200	2	10	20	100	200	500	1,000	
MoE	One-Generation Study	C*	P*	P*	C*,P*						A*		Oral42days
METI	Uterotrophic Assay estrogenic						–		–			–	Oral,7days, ovariectomized
	Uterotrophic Assay antiestrogenic						–		–			–	Oral,7days, ovariectomized
	Uterotrophic Assay					–		–		–			subcutaneous,3days
	Hershberber Assay androgenic						–		–			–	Oral10days, gonadectomized
	Hershberber Assay antiandrogenic						–		–			–	Oral10days, gonadectomized
	Two-Generation Study												Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present(pending).

Results of METI (– :negative, + :positive)

<Signs of A*>

_ F_0 dams: decreases in body weight, food consumption and the number of litter, increases in absolute and relative weights of the liver and adrenals, hypertrophy of the centrilobular hepatocyte and a prolongation of gestation period.

_ F_1 male offspring: decreases in body weight, absolute and relative weights of the testes, seminal vesicle, epididymides, kidneys, prostate and levator ani muscle, defects of the kidney, epididymis, ureter and seminal vesicle, small testis and small epididymis, hypoplasia/agenesis of the epididymis, disappearance of the germ cell in the seminiferous tubule, hyperplasia of the leydig cell, formation of the giant cell in the seminiferous tubule, disappearance of the sperm in the lumen of the epididymis, cell debris in the lumen of the epididymis and increase of the mRNA expression in AR in the prostate.

_ F_1 female offspring: decrease in body weight, small uterus, hypoplasia/agenesis of the uterine horn, defects of the kidney, ureter, ovary, oviduct and uterine horn, and mineralization of the corticomedullary junction in the kidney.

(The underline showed the view that have already been reported.)

<Signs of C*>

_ F_0 dams: decrease in the number of implantations

• F_1 male offspring: decreases in the absolute and relative pituitary weights in the 1.6 μg/kg.

_ F_1 female offspring: increase in dead embryo/fetus indices in the 1.6 μg/kg

<Signs of P*>

_ F_1 female offspring: increase in mRNA expression in ERα of the uterus and AR of the prostate in the 8, 40 and 200μg/kg.

ANNEX 5-5 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent(continued)

Di-cyclohexyl phthalate

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range	Comment
MoE	ERα binding assay	18% at 4.5 x 10^{-5}M	10^{-9} –4.5 x 10^{-5}M	
	ERβ binding assay	9% at 4.5 x 10^{-5}M	10^{-9} – 10^{-4}M	
	E-screen	15% of E2 at 10^{-5}M	10^{-9} – 10^{-4}M	cell toxicity at 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} – 10^{-5}M	
	AR reporter gene assay (antagonist)	–	10^{-6} – 10^{-5}M	
	AR binding assay	9% at 10^{-4}M	10^{-6} – 10^{-3}M	
	TRα	–	<2 x 10^{-5}M	
	TRβ	–	<2 x 10^{-5}M	

–: indicates no activity

ANNEX 5-6 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Di-(2-ethylhexyl) phthalate

(1) ***in vivo*** **Tests**

competent authorities	Dose Assay and Test	μg/kg/day			mg/kg/day		Comment
		10	50	250	1.25	100	
MoE	One-Generation Study	D	C F1 female: Increase of serum concentration of FSH	D	D	A F0 dam: Enlargement of the liver, Increase of the liver weight*; Centrilobular hypertrophy of the hepatocyte*; Increase of eosinophilic granule of the hepatocyte (*: Findings reported in other studies)	Oral 42 days
METI	Hershberber Assay						Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI (– : negative, + : positive)

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range	Comment
MoE	ERα binding assay	17% at 2.2×10^{-6}M	10^{-11}–4.5×10^{-6}M	
	ERβ binding assay	13% at 2.2×10^{-6}M	10^{-11} – 4.5×10^{-6}M	
	E-screen	–	10^{-9} – 10^{-4}M	cell toxicity at 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} – 10^{-5}M	
	AR reporter gene assay(antagonist)	–	10^{-6} – 10^{-5}M	
	AR binding assay	1% at 3.0×10^{-5}M	10^{-6} – 10^{-3}M	
	TRα	–	<2 – 10^{-5}M	
	TRβ	–	<2 – 10^{-5}M	

– : indicates no activity

ANNEX 5-7 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Butylbenzyl phthalate

(1) ***in vivo*** **Tests**

competent authorities	Dose / Assay and Test	μg/kg/day				mg/kg/day			g/kg/day		Comment
		2	12	60	300	40	200	500	1	2	
MoE	One-Generation Study	D	D	P	P			A			Oral42days
METI	Uterotrophic Assay(estrogenic)							–	–	–	subcutaneous,3days, immature
	Uterotrophic Assay(antiestrogenic)							–	–	–	subcutaneous,3days, immature
	Hershberber Assay(androgenic)					–	–		–		Oral10days,gonadectomized
	Hershberber Assay(antiandrogenic)					–	+		–		Oral10days,gonadectomized
	Two-Generation Study										Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported.
However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI (– : negative, + :positive)

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range
MoE	ERα binding assay	38% at 10^{-4}M	10^{-11} – 10^{-4}M
	ERβ binding assay	23% at 10^{-4}M	10^{-11} – 10^{-4}M
	E-screen	–	10^{-9} – 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} – 10^{-5}M
	AR reporter gene assay(antagonist)	IC_{50}=3.5 x 10^{-5}M	10^{-6} – 10^{-4}M
	AR binding assay	IC_{50}=2.1 x 10^{-5}M	10^{-5} – 10^{-3}M
	TRα	–	<2 x 10^{-5}M
	TRβ	–	<2 x 10^{-5}M

– : indicates no activity

ANNEX 5-8 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Diethyl phthalate

(1) ***in vivo*** **Tests**

Competent authorities	Dose Assay and Test	μg/kg/day				mg/kg/day			Comment
		0.4	2	10	50	200	600	2,000	
MoE	One-Generation Study	C F_0 dams: A decrease in pituitary weight (absolute & relative)	C F_0 dams: A decrease in pituitary weight (absolute & relative)	C F_0 dams: Decreases in pituitary weight (absolute & relative) and thyroid weight (absolute & relative)	C F_0 dams: Decreases in pituitary weight (absolute & relative) and thyroid weight (absolute & relative) F_1 male: Delay of preputial separation			A F_0 dams[1)] F_1 pups[2)] F_1 males[3)] F_1 females[4)]	Oral 42days
METI	Uterotrophic Assay estrogenic					–	–	–	Subcutaneous 7days ovariectomized
	Uterotrophic Assay antiestrogenic					–	–	–	subcutaneous 7days ovariectomized
	Hershberber Assay androgenic					–	–	–	Oral10days, gonadectomized
	Hershberber Assay antiandrogenic					–	–	–	Oral10days, gonadectomized
	Two-Generation Study								Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported.
However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI (– : negative, + : positive)

[1)] F_0 dams: Decreases in body weight, body weight gain, food consumption, pituitary weight (absolute and relative) and thyroid weight (absolute and relative); and eosinophilic, granular change of liver.
[2)] F_1 pups: Decreases in viability index and number of live offspring.
[3)] F_1 males: Decreases in body weight, body weight gain, sperm motility (straight line velocity), thymus weight (absolute and relative) and testis weight (absolute and relative); delays of behavioral development (negative geotaxis), physical development (ear opening and eye opening) and preputial separation; changed FSH level in serum (decreased; day 21 of lactation, increased; after mating), and histopathological changes in the testis (day 21 of lactation: decreased number of the germ cells and appearance of the elongated nuclear cells, after weaning: focal tubular atrophy).
[4)] F_1 females: Decreases in body weight, body weight gain, thymus weight (absolute and relative), kidney weight (absolute and relative) and motor activity (horizontal movement and vertical movement); an increase in AGD (absolute and relative); delays of behavioral development (cliff aversion and negative geotaxis) and physical development (ear opening, eye opening).
(The underlined findings have already been reported.)

Diethyl phthalate

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range
MoE	ERα bindina assay	3% at 4.5 x 10^{-7}M	10^{-11} – 10^{-4}M
	ERβ bindina assay	3% at 10^{-4}M	10^{-11} – 10^{-4}M
	E-screen	7% of E2 at 10^{-4}M	10^{-11} – 10^{-4}M
	Arreporter gene assay (agonist)	–	10^{-6} – 10^{-5}M
	Arreporter gene assay (antagonist)	IC_{50}=7.9 x 10^{-5}M	10^{-6} – 10^{-5}M
	Arbinding assay	IC_{50}=1.5 x 10^{-3}M	10^{-5} – 10^{-3}M
	TRα	–	<10^{-4}M
	TRβ	–	<10^{-4}M

– : indicates no activity

ANNEX 5-9 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Di-(2-ethylhexyl) adipate

(1) ***in vivo*** **Tests**

competent authorities	Dose / Assay and Test	µg/kg/day		mg/kg/day			Comment
		15	150	1.5	15	600	
MoE	One-Generation Study	D	C F0 dam: low body weight gain	C F1 male: low testosterone in serum	C F1 male: low testosterone in serum	A*	Oral 42days
METI	Uterotrophic Assay						Being conducted
	Hershberber Assay						Being conducted

A: Substantial changes(statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related(suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present(pending).
Results of METI (–: negative, + : positive)
<*Findings in A>
F0 Dam: high liver weight (absolute and relative).
F1 Pup: high number of stillbirths; low weaning rate.
F1 Male: low testosterone in serum.
F1 Female: low ER á mRNA in the ovaries.
(Under line: recognized in other reports)

(2) ***in vitro*** **Assay**

competent authorities	Assay	Results	Range
MoE	ERα binding assay	8% at 4.5 x 10^{-7}M	10^{-11} – 10^{-4}M
	ERβ binding assay	3% at 10^{-6}M	10^{-11} – 10^{-4}M
	E-screen	–	10^{-9} – 10^{-4}M
	AR reporter gene assay(agonist)	–	10^{-6} – 10^{-5}M
	AR reporter gene assay(antagonist)	–	10^{-6} – 10^{-5}M
	AR binding assay	13% at 10^{-3}M	10^{-5} – 10^{-3}M
	TRα	–	<10^{-4}M
	TRβ	–	<10^{-4}M

– : indicates no activity

ANNEX 5-10 Results of Assays and Tests in Evaluation of the Endocrine Disrupting Activities in Rodent

Triphenyltin chloride

(1) *in vivo* Tests

Competent authorities	Dose Assay and Test	μg/kg/day				mg/kg/day	Comment
		1.1	11	107	370	1.117	
		0.015	0.15	1.5	5	15	ppm
MoE	One-Generation Study	D	D	C F1 males: Low values of body weight	C F0 dams: High values of food efficiency P F1 females: Low values of blood concentration of T_3	A*	Diet 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.
B: Treatment-related effects (significant differences from the control) were observed below the LOEL or LOAEL at which some effects have already been reported.
C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.
D: No statistically significant difference from the control was observed.
S: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. Additional tests have currently been conducted to confirm whether the alterations are treatment-related (suspended).
P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).
Results of METI (–: negative, + : positive)
<Findings observed at A*>
*F0 dams: Low values of food consumption.
*F1 pups: Low values of viability.
(The underlined findings are those reported in certain animals.)

(2) *in vitro* Assay

Competent authorities	Assay	Results	Range	Comment
MoE	ERα binding assay	38% at 2.2×10^{-5}M	10^{-11}–2.2×10^{-5}M	
	ERβ binding assay	13% at 1.8×10^{-5}M	2.2×10^{-11} – 10^{-4}M	
	E-screen	–	10^{-14} – 10^{-4}M	cell toxicity at 10^{-9}– 10^{-4}M
	AR reporter gene assay (agonist)	–	10^{-6} – 10^{-5}M	cell toxicity at $>0.3 \times 10^{-6}$M
	AR reporter gene assay (antagonist)	–	10^{-6} – 10^{-5}M	cell toxicity at $>0.3 \times 10^{-6}$M
	AR binding assay	$IC_{50}=1.3 \times 10^{-4}$M	10^{-5} – 10^{-3}M	difficult to distinguish from cell toxicity
	TRα	–	$<2 \times 10^{-5}$M	
	TRβ	–	$<2 \times 10^{-5}$M	

IC_{50} (the concentration that inhibits 50% combination of labeled hormone and receptor), PC_{50} (the concentration that induces 50% activity of chemiluminescent intensity induced by E2, etc.), Ecx_{10} (the concentration that shows chemiluminescent intensity of 10 times as much as the background value) obtained were recorded. When these values were not obtained, the maximum reaction value and the concentration were recorded.
Values were analyzed for statistically significant differences, and –indicates no activity.