AOP ID and Title:

SNAPSHOT

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AOP 64: Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility

Short Title: Adult Leydig Cell Dysfunction

Authors

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite		1.29	Under Development

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	494	Glucocorticoid Receptor Agonist, Activation (https://aopwiki.org/events/494)	GR Agonist, Activation
2	KE	495	Repressed expression of steroidogenic enzymes (https://aopwiki.org/events/495)	Repressed expression of steroidogenic enzymes
3	KE	496	Increased apoptosis, decreased number of adult Leydig Cells (https://aopwiki.org/events/496)	Increased apoptosis, decreased Leydig Cells
4	KE	413	Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/events/413)	Reduction, Testosterone synthesis in Leydig cells
5	KE	446	Reduction, testosterone level (https://aopwiki.org/events/446)	Reduction, testosterone level
6	KE	520	Decreased sperm quantity or quality in the adult, Decreased fertility (https://aopwiki.org/events/520)	Decreased sperm quantity or quality in the adult, Decreased fertility
7	AO	406	impaired, Fertility (https://aopwiki.org/events/406)	impaired, Fertility

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Glucocorticoid Receptor Agonist, Activation (https://aopwiki.org/relationships/1645)	adjacent	Increased apoptosis, decreased number of adult Leydig Cells		

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Glucocorticoid Receptor Agonist, Activation (https://aopwiki.org/relationships/1646)	adjacent	Repressed expression of steroidogenic enzymes		
Increased apoptosis, decreased number of adult Leydig Cells (https://aopwiki.org/relationships/1647)	adjacent	Reduction, Testosterone synthesis in Leydig cells		
Repressed expression of steroidogenic enzymes (https://aopwiki.org/relationships/1648)	adjacent	Reduction, Testosterone synthesis in Leydig cells		
Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/relationships/439)	adjacent	Reduction, testosterone level		
Reduction, testosterone level (https://aopwiki.org/relationships/1649)	adjacent	Decreased sperm quantity or quality in the adult, Decreased fertility		
Decreased sperm quantity or quality in the adult, Decreased fertility (https://aopwiki.org/relationships/1650)	adjacent	impaired, Fertility		

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Sex Applicability

Sex	Evidence
Male	

References

Appendix 1

List of MIEs in this AOP

Event: 494: Glucocorticoid Receptor Agonist, Activation (https://aopwiki.org/events/494)

Short Name: GR Agonist, Activation

Key Event Component

Process	Object	Action
glucocorticoid receptor activity	glucocorticoid receptor	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	MolecularInitiatingEvent

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
Leydig cell

List of Key Events in the AOP

Event: 495: Repressed expression of steroidogenic enzymes (https://aopwiki.org/events/495)

Short Name: Repressed expression of steroidogenic enzymes

Key Event Component

Process	Object	Action
hormone biosynthetic process	testosterone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
Leydig cell

Event: 496: Increased apoptosis, decreased number of adult Leydig Cells (https://aopwiki.org/events/496)

Short Name: Increased apoptosis, decreased Leydig Cells

Key Event Component

Process	Object	Action
apoptotic process		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
Leydig cell

Event: 413: Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/events/413)

Short Name: Reduction, Testosterone synthesis in Leydig cells

Key Event Component

Process	Object	Action
testosterone biosynthetic process	testosterone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:51 - PPARα activation leading to impaired fertility in adult male rodents (https://aopwiki.org/aops/51)	KeyEvent
Aop:18 - PPARα activation in utero leading to impaired fertility in males (https://aopwiki.org/aops/18)	KeyEvent
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
testosterone secreting cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Term	Scientific Term	Evidence	Links
mice	Mus sp.	Low	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10095)

Key enzymes needed for testosterone production first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). Consequently, this key event is applicable to most vertebrates, including humans.

Key Event Description

Biological state

Testosterone is a steroid hormone from the androgen group and is found in humans and other vertebrates.

Biological compartments

In humans and other mammals, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females and other steroidogenic tissues (e.g., brain, adipose). It either acts locally /or is transported to other tissues via blood circulation. Testosterone synthesis takes place within the mitochondria of Leydig cells, the testosterone-producing cells of the testis. It is produced upon stimulation of these cells by Luteinizing hormone (LH) that is secreted in pulses into the peripheral circulation by the pituitary gland in response to Gonadotropin-releasing hormone (GnRH) from the hypothalamus. Testosterone and its aromatized product, estradiol, feed back to the hypothalamus and pituitary gland to suppress transiently LH and thus testosterone production. In response to reduced testosterone levels, GnRH and LH are produced. This negative feedback cycle results in pulsatile secretion of LH followed by pulsatile production of testosterone (Ellis, Desjardins, and Fraser 1983), (Chandrashekar and Bartke 1998).

General role in biology

Testosterone is the principal male sex hormone and an anabolic steroid. Male sexual differentiation depends on testosterone (T), dihydrotestosterone (DHT), and the expression of androgen receptors by target cells (Manson and Carr 2003). During the development secretion of androgens by Leydig cells is essential for masculinization of the foetus (Nef 2000). The foetal Leydig cells develop in utero. These cells become competent to produce testosterone in rat by gestational day (GD) 15.5, with increasing production thereafter. Peak steroidogenic activity is reached just prior to birth, on GD19 (Chen, Ge, and Zirkin 2009). Testosterone secreted by foetal Leydig cells is required for the differentiation of the male urogenital system late in gestation (Huhtaniemi and Pelliniemi 1992). Foetal Leydig cells also play a role in the scrotal descent of the testis through their synthesis of insulin-like growth factor 3 (Insl3), for review see (Nef 2000).

In humans, the first morphological sign of testicular differentiation is the formation of testicular cords, which can be seen between 6 and 7 weeks of gestation. Steroid-secreting Leydig cells can be seen in the testis at 8 weeks of gestation. At this period, the concentration of androgens in the testicular tissue and blood starts to rise, peaking at 14-16 weeks of gestation. This increase comes with an increase in the number of Leydig cells for review see (Rouiller-Fabre et al. 2009).

Adult Leydig cells, which are distinct from the foetal Leydig cells, form during puberty and supply the testosterone required for the onset of spermatogenesis, among other functions. Distinct stages of adult Leydig cell development have been identified and characterized. The stem Leydig cells are undifferentiated cells that are capable of indefinite self-renewal but also of differentiation to steroidogenic cells. These cells give rise to progenitor Leydig cells, which proliferate, continue to differentiate, and give rise to the immature Leydig cells. Immature Leydig cells synthesize high levels of testosterone metabolites and develop into terminally differentiated adult Leydig cells, which produce high levels of testosterone. With aging, both serum and testicular testosterone concentrations progressively decline, for review see (Nef 2000).

Androgens play a crucial role in the development and maintenance of male reproductive and sexual functions. Low levels of circulating androgens can cause disturbances in male sexual development, resulting in congenital abnormalities of the male reproductive tract. Later in life, this may cause reduced fertility, sexual dysfunction, decreased muscle formation and bone mineralisation, disturbances of fat metabolism, and cognitive dysfunction. Testosterone levels decrease as a process of ageing: signs and symptoms caused by this decline can be considered a normal part of ageing.

How it is Measured or Detected

OECD TG 456 [1] (http://www.oecd-ilibrary.org/environment/test-no-456-h295r-steroidogenesis-assay_9789264122642-en) is the validated test guideline for an in vitro screen for chemical effects on steroidogenesis, specifically the production of 17ß-estradiol (E2) and testosterone (T). The testosterone syntheis can be measured in vitro cultured Leydig cells. The methods for culturing Leydig cells can be found in the Database Service on Alternative Methods to animal experimentation (DB-ALM): Leydig Cell-enriched Cultures [2] (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_met=232), Testicular Organ and Tissue Culture Systems [3] (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_met=515).

Testosterone synthesis in vitro cultured cells can be measured indirectly by testosterone radioimmunoassay or analytical methods such as LC-MS.

References

Chandrashekar, V, and A Bartke. 1998. “The Role of Growth Hormone in the Control of Gonadotropin Secretion in Adult Male Rats.” Endocrinology 139 (3) (March): 1067–74. doi:10.1210/endo.139.3.5816.

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Nef, S. 2000. “Hormones in Male Sexual Development.” Genes & Development 14 (24) (December 15): 3075–3086. doi:10.1101/gad.843800.

Rouiller-Fabre, Virginie, Vincent Muczynski, Romain Lambrot, Charlotte Lécureuil, Hervé Coffigny, Catherine Pairault, Delphine Moison, et al. 2009. “Ontogenesis of Testicular Function in Humans.” Folia Histochemica et Cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society 47 (5) (January): S19–24. doi:10.2478/v10042-009-0065-4.

Event: 446: Reduction, testosterone level (https://aopwiki.org/events/446)

Short Name: Reduction, testosterone level

Key Event Component

Process	Object	Action
	testosterone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:51 - PPARα activation leading to impaired fertility in adult male rodents (https://aopwiki.org/aops/51)	KeyEvent
Aop:18 - PPARα activation in utero leading to impaired fertility in males (https://aopwiki.org/aops/18)	KeyEvent
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
blood

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Key enzymes needed for testosterone production first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). Consequently, this key event is applicable to most vertebrates, including humans.

Key Event Description

Biological state

Testosterone (T) is a steroid hormone from the androgen group. T serves as a substrate for two metabolic pathways that produce antagonistic sex steroids.

Biological compartments

Testosterone is synthesized by the gonads and other steroidogenic tissues (e.g., brain, adipose), acts locally and/or is transported to other tissues via blood circulation. Leydig cells are the testosterone-producing cells of the testis.

General role in biology

Androgens, the main male sex steroids, are the critical factors responsible for the development of the male phenotype during embryogenesis and for the achievement of sexual maturation at puberty. In adulthood, androgens remain essential for the maintenance of male reproductive function and behaviour. Apart from their effects on reproduction, androgens affect a wide variety of non-reproductive tissues such as skin, bone, muscle, and brain (Heemers, Verhoeven, & Swinnen, 2006). Androgens, principally T and 5α-dihydrotestosterone (DHT), exert most of their effects by interacting with a specific receptor, the androgen receptor (AR), for review see (Murashima, Kishigami, Thomson, & Yamada, 2015). On the one hand, testosterone can be reduced by 5α-reductase to produce 5α dihydrotestosterone (DHT). On the other hand, testosterone can be aromatized to generate estrogens. Testosterone effects can also be classified by the age of usual occurrence, postnatal effects in both males and females are mostly dependent on the levels and duration of circulating free testosterone.

How it is Measured or Detected

Testosterone can be measured by immunoassays and by isotope-dilution gas chromatography-mass spectrometry in serum (Taieb et al., 2003), (Paduch et al., 2014). Testosterone levels are measured i.a. in: Fish Lifecycle Toxicity Test (FLCTT) (US EPA OPPTS 850.1500), Male pubertal assay (PP Male Assay) (US EPA OPPTS 890.1500), OECD TG 441: Hershberger Bioassay in Rats (H Assay).

References

Heemers, H. V, Verhoeven, G., & Swinnen, J. V. (2006). Androgen activation of the sterol regulatory element-binding protein pathway: Current insights. Molecular Endocrinology (Baltimore, Md.), 20(10), 2265–77. doi:10.1210/me.2005-0479

Murashima, A., Kishigami, S., Thomson, A., & Yamada, G. (2015). Androgens and mammalian male reproductive tract development. Biochimica et Biophysica Acta, 1849(2), 163–170. doi:10.1016/j.bbagrm.2014.05.020

Paduch, D. A., Brannigan, R. E., Fuchs, E. F., Kim, E. D., Marmar, J. L., & Sandlow, J. I. (2014). The laboratory diagnosis of testosterone deficiency. Urology, 83(5), 980–8. doi:10.1016/j.urology.2013.12.024

Taieb, J., Mathian, B., Millot, F., Patricot, M.-C., Mathieu, E., Queyrel, N., … Boudou, P. (2003). Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clinical Chemistry, 49(8), 1381–95.

Event: 520: Decreased sperm quantity or quality in the adult, Decreased fertility (https://aopwiki.org/events/520)

Short Name: Decreased sperm quantity or quality in the adult, Decreased fertility

Key Event Component

Process	Object	Action
sperm quantity		decreased
	sperm	morphological change
	fertility	decreased
	sperm	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:70 - Modulation of Adult Leydig Cell Function Subsequent to Proteomic Alterations in the Adult Leydig Cell (https://aopwiki.org/aops/70)	AdverseOutcome
Aop:71 - Modulation of Adult Leydig Cell Function Subsequent to Glucocorticoid Activation (https://aopwiki.org/aops/71)	AdverseOutcome
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	KeyEvent

Biological Context

Level of Biological Organization
Individual

List of Adverse Outcomes in this AOP

Event: 406: impaired, Fertility (https://aopwiki.org/events/406)

Short Name: impaired, Fertility

Key Event Component

Process	Object	Action
fertility		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:7 - Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female (https://aopwiki.org/aops/7)	AdverseOutcome
Aop:51 - PPARα activation leading to impaired fertility in adult male rodents (https://aopwiki.org/aops/51)	AdverseOutcome
Aop:18 - PPARα activation in utero leading to impaired fertility in males (https://aopwiki.org/aops/18)	AdverseOutcome
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	AdverseOutcome

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Key Event Description

Biological state

capability to produce offspring

Biological compartments

System

General role in biology

Fertility is the capacity to conceive or induce conception. Impairment of fertility represents disorders of male or female reproductive functions or capacity.

How it is Measured or Detected

As a measure, fertility rate, is the number of offspring born per mating pair, individual or population.

Regulatory Significance of the AO

Under REACH, information on reproductive toxicity is required for chemicals with an annual production/importation volume of 10 metric tonnes or more. Standard information requirements include a screening study on reproduction toxicity (OECD TG 421/422) at Annex VIII (10-100 t.p.a), a prenatal developmental toxicity study (OECD 414) on a first species at Annex IX (100-1000 t.p.a), and from March 2015 the OECD 443(Extended One-Generation Reproductive Toxicity Study) is reproductive toxicity requirement instead of the two generation reproductive toxicity study (OECD TG 416). If not conducted already at Annex IX, a prenatal developmental toxicity study on a second species at Annex X (≥ 1000 t.p.a.).

Under the Biocidal Products Regulation (BPR), information is also required on reproductive toxicity for active substances as part of core data set and additional data set (EU 2012, ECHA 2013). As a core data set, prenatal developmental toxicity study (EU TM B.31) in rabbits as a first species and a two-generation reproduction toxicity study (EU TM B.31) are required. OECD TG 443 (Extended One-Generation Reproductive Toxicity Study) shall be considered as an alternative approach to the multi-generation study.) According to the Classification, Labelling and Packaging (CLP) regulation (EC, 200; Annex I: 3.7.1.1): a) “reproductive toxicity” includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring; b) “effects on fertility” includes adverse effects on sexual function and fertility; and c) “developmental toxicity” includes adverse effects on development of the offspring.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1645: GR Agonist, Activation leads to Increased apoptosis, decreased Leydig Cells (https://aopwiki.org/relationships/1645)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Relationship: 1646: GR Agonist, Activation leads to Repressed expression of steroidogenic enzymes (https://aopwiki.org/relationships/1646)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Relationship: 1647: Increased apoptosis, decreased Leydig Cells leads to Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/relationships/1647)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Relationship: 1648: Repressed expression of steroidogenic enzymes leads to Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/relationships/1648)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Relationship: 439: Reduction, Testosterone synthesis in Leydig cells leads to Reduction, testosterone level (https://aopwiki.org/relationships/439)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
PPARα activation in utero leading to impaired fertility in males (https://aopwiki.org/aops/18)	**adjacent**	**High**	
PPARα activation leading to impaired fertility in adult male rodents (https://aopwiki.org/aops/51)	**adjacent**	**High**	
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
mice	Mus sp.	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10095)

Ses Table 1.

Key Event Relationship Description

Impairment of testosterone production in testes directly impacts on testosterone levels.

Evidence Supporting this KER

Biological Plausibility

Within the testes, steroid synthesis takes place within the mitochondria of Leydig cells. Testosterone production by Leydig cells is primarily under the control of LH. LH indirectly stimulates the transfer of cholesterol into the mitochondrial matrix to cholesterol side-chain cleavage cytochrome P450 (P450scc, CYP11A), which converts cholesterol to pregnenolone. Pregnenolone diffuses to the smooth endoplasmic reticulum where it is further metabolized to testosterone via the actions of 3β-hydroxysteroid dehydrogenase Δ5-Δ4-isomerase (3β-HSD), 17α-hydroxylase/C17-20 lyase (P450c17, CYP17), and 17β-hydroxysteroid dehydrogenase type III (17HSD3). For review see (Payne & Hales, 2013). Therefore, inhibition or impairment of the testosterone production directly impacts on the levels of testosterone.

Empirical Evidence

There is evidence from experimental work that demonstrates a coordinated, dose-dependent reduction in the production of testosterone and consecutive reduction of testosterone levels in foetal testes and in serum, see Table 1.

			KE: testosterone synthesis, reduction	**KE: testosterone, reduction**		
Compound	Species	Effect level			Details	References
Phthalates (DEHP)	rat	LOEL =300 mg/kg/day	testicular testosterone production, reduction (ex vivo)	testicular testosterone levels, reduction, no change plasma testosterone	testosterone levels at GD 21 in male rat fetuses exposed to 0, 10, 30, 100, or 300 mg /kg bw/day from GD 7 to GD 21 testicular testosterone production ex vivo	(Borch, Metzdorff, Vinggaard, Brokken, & Dalgaard, 2006)
Phthalates (DBP)	rat	LOEL =50 mg/kg/day		testicular testosterone levels, reduction,	Testicular testosterone was reduced >50 mg/kg/day	(Shultz, 2001)
Phthalates (DEHP)	rat	LOEL=300 mg/kg/day	fetal testicular testosterone production, reduction			(Borch, Ladefoged, Hass, & Vinggaard, 2004)
Phthalates (DEHP)	rat	LOEL=300 mg/kg/day		testicular testosterone levels, reduction,		(Borch et al., 2004)
Phthalates (DEHP)	rat	LOEL=300 mg/kg/day		No change plasma testosterone		(Borch et al., 2004)
Phthalates (DEHP)	rat	LOEL=100 mg/kg/day		Serum testosterone levels, reduction,		(Akingbemi, 2001)
Phthalates (DEHP)	rat	LOEL=750 mg /kg /day		testicular testosterone levels, reduction, by 60 – 85%		(Parks, 2000)
Phthalates (DEHP)	rat	LOEL=750 mg /kg/day		testosterone levels, reduction, fetuses on GD 17 (71% lower than controls) and 18 (47% lower than controls)		(Parks, 2000)
Phthalates (DEHP)	rat	LOEL=750mg/kg/day	ex vivo testosterone production, reduction by 50%			(Wilson et al., 2004)
Phthalates (DEHP)	rat	LOEL=234 mg/kg/day		serum testosterone levels, reduction,		(Culty et al., 2008)
Phthalates (DEHP)	rat	LOEL=1250 mg/kg/day	*ex vivo* foetal testicular production			(Culty et al., 2008)
Phthalates (DEHP)	rat	ED50=444,2 mg/kg/day	*ex vivo* foetal testicular production, reduction			(Hannas et al., 2012)

Phthalates (DHP)	rat	ED50=75.25 mg/kg/day	*ex vivo* foetal testicular production, reduction			(Hannas et al., 2012)

Table 1. Summary table for empirical support for this KER. ED50 - half maximal effective concentration, LOEL- lowest observed effect level, Dibutyl phthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP), Dihexyl Phthalate (DHP).

References

Akingbemi, B. T. 2001. “Modulation of Rat Leydig Cell Steroidogenic Function by Di(2-Ethylhexyl)Phthalate.” Biology of Reproduction 65 (4) (October 1): 1252–1259. doi:10.1095/biolreprod65.4.1252.

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Relationship: 1649: Reduction, testosterone level leads to Decreased sperm quantity or quality in the adult, Decreased fertility (https://aopwiki.org/relationships/1649)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		

Relationship: 1650: Decreased sperm quantity or quality in the adult, Decreased fertility leads to impaired, Fertility (https://aopwiki.org/relationships/1650)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64)	**adjacent**		