AOP ID and Title:

SNAPSHOT

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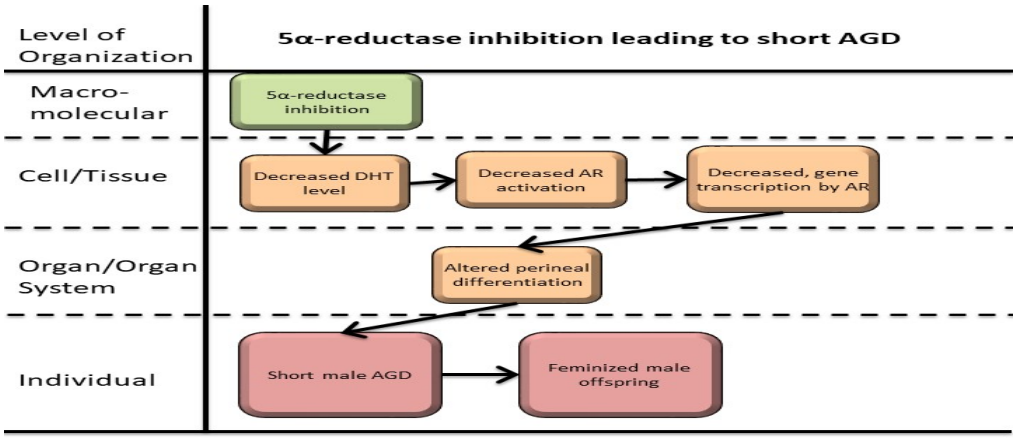
AOP 305: 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring

Short Title: 5α-reductase inhibition leading to short AGD

Graphical Representation



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Status

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Abstract

This AOP links 5α-reductase inhibition during fetal life with short anogenital distance (AGD) in male offspring. A short AGD around birth is a marker for feminization of male fetuses and is associated with male reproductive disorders, including reduced fertility in adulthood. Although a short AGD is not necessarily ‘adverse’ from a human health perspective, it is considered an ‘adverse outcome’ in OECD test guidelines; AGD measurements are mandatory in specific tests for developmental and reproductive toxicity in chemical risk assessment (TG 443, TG 421/422, TG 414).

5α-reductase is an enzyme responsible for the conversion of testosterone to DHT in target tissues. DHT is more potent agonist of the Androgen receptor (AR) than testosterone, so that DHT is necessary for proper masculinization of e.g. male external genitalia. Under normal physiological conditions, testosterone produced mainly by the testicles, is converted in peripheral tissues by 5α-reductase into DHT, which in turn binds AR and activates downstream target genes. AR signaling is necessary for masculinization of the developing fetus, including differentiation of the levator ani/bulbocavernosus (LABC) muscle complex in males. The LABC complex does not develop in the absence, or low levels of, androgen signaling, as in female fetuses.

The key events in this pathway is inhibition of 5α-reductase that converts testosterone into the more potent DHT in androgen sensitive target tissues. This includes developing perineal region, which, when DHT levels are low or absent, leads to inactivation of the AR and failure to properly masculinize the perineum/LABC complex.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
	MIE	1617	5α-reductase, inhibition (https://aopwiki.org/events/1617)	5α-reductase, inhibition
	KE	1613	Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/events/1613)	Decrease, DHT level
	KE	1614	Decrease, androgen receptors (AR) activation (https://aopwiki.org/events/1614)	Decrease, AR activation
	KE	1687	decrease, transcription of genes by AR (https://aopwiki.org/events/1687)	decrease, transcription of genes by AR
	AO	1688	decrease, male anogenital distance (https://aopwiki.org/events/1688)	short male AGD

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
5α-reductase, inhibition (https://aopwiki.org/relationships/1880)	adjacent	Decrease, dihydrotestosterone (DHT) level	High	High
Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/relationships/1935)	adjacent	Decrease, androgen receptors (AR) activation	High	Moderate
Decrease, androgen receptors (AR) activation (https://aopwiki.org/relationships/2128)	adjacent	decrease, transcription of genes by AR	Moderate	Low
decrease, transcription of genes by AR (https://aopwiki.org/relationships/2129)	adjacent	decrease, male anogenital distance	Moderate	Low

Stressors

Name	Evidence
Finasteride	High

Finasteride

Finasteride is a type II 5alpha-reductase inhibitor that blocks conversion of testosterone to dihydrotestosterone (Clark et al 1990; Imperato-McGinley et al 1992). Intrauterine exposure in rats can result in shorter male AGD in male offspring (Bowman et al 2003; Christiansen et al 2009; Schwartz et al 2019)

References:

Bowman et al (2003), Toxicol Sci 74:393-406; doi: 10.1093/toxsci/kfg128

Christiansen et al (2009), Environ Health Perspect 117:1839-1846; doi: 10.1289/ehp.0900689

Clark et al (1990), Teratology 42:91-100; doi: 10.1002/tera.1420420111

Imperato-McGinley (1992), J Clin Endocrinol Metab 75:1022-1026; doi: 10.1210/jcem.75.4.1400866

Schwartz et al (2019), Toxicol Sci 169:303-311; doi: 10.1093/toxsci/kfz046

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Pregnancy	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	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	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Sex Applicability

Sex	Evidence
Male	High

References

1. Schwartz CL, Christiansen S, Vinggaard AM, Axelstad M, Hass U and **Svingen T** (2019), Anogenital distance as a toxicological or clinical marker for fetal androgen action and risk for reproductive disorders. *Arch Toxicol* 93: 253-272.

Appendix 1

List of MIEs in this AOP

Event: 1617: 5α-reductase, inhibition (https://aopwiki.org/events/1617)

Short Name: 5α-reductase, inhibition

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	MolecularInitiatingEvent
Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	MolecularInitiatingEvent

Biological Context

Level of Biological Organization
Molecular

List of Key Events in the AOP

Event: 1613: Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/events/1613)

Short Name: Decrease, DHT level

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:288 - Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288)	KeyEvent
Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	KeyEvent
Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	KeyEvent
Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Key Event Description

Reduction in DHT synthesis leads to a reduction in DHT circulating levels. [1][2]

How it is Measured or Detected

DHT levels in a sample can be measured by (High Performance) Liquid Chromatography. After sample fractionation, DHT can be identify by comparison with internal standards spectrum. Quantification of DHT levels can be performed using hormones measurements kits (ELISA), instrumental techniques (LC-MS) or liquid scintillation spectrometry (after radiolabeling).[3]

References

[1] Miller Walter L. (1988) Molecular Biology of Steroid Hormone Synthesis. Endocrine Reviews, 9(3): 295-318.https://doi.org/10.1210/edrv-9-3-295 (https://www.google.com/url?q=https://doi.org/10.1210/edrv-9-3-295&sa=D&ust=1554891396614000)

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Event: 1614: Decrease, androgen receptors (AR) activation (https://aopwiki.org/events/1614)

Short Name: Decrease, AR activation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:288 - Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288)	KeyEvent
Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	KeyEvent
Aop:306 - Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306)	KeyEvent
Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)	KeyEvent
Aop:344 - Androgen receptor (AR) antagonism leading to nipple retention (NR) in male (mammalian) offspring (https://aopwiki.org/aops/344)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Key Event Description

Androgen receptor activation is regulated by the binding of androgens. AR activity can be decreased by either a lack of steroidal ligands (testosterone, DHT) or the presence of antagonist compounds. [1][2]

How it is Measured or Detected

Significance of AR signaling in fetal development can be studied through a conditional deletion of the androgen receptor using a Cre/loxP approach. The recommended animal model for reproductive study is the mouse.[3]

Also, epidemiological case-studies following mouse or humans expressing a complete androgen insensitivity allow to directly assess the effects of a lack of AR activation on the development.[4]

Enzyme immunoassay (ELISA) kits for in vitro quantitative measurement of AR activity are available. Androgen receptors activity can be measured using bioassay such as the (Anti-)Androgen Receptor CALUX reporter gene assay.[5]

References

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[2] Gao W., Bohl C.E. and Dalton J.T. (2005) Chemistry and Structural Biology of Androgen Receptor. Chemical Reviews 105(9): 3352-3370https://doi.org/10.1021/cr020456u (https://www.google.com/url?q=https://doi.org/10.1021/cr020456u&sa=D&ust=1554891396627000)

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Event: 1687: decrease, transcription of genes by AR (https://aopwiki.org/events/1687)

Short Name: decrease, transcription of genes by AR

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	KeyEvent
Aop:306 - Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

List of Adverse Outcomes in this AOP

Event: 1688: decrease, male anogenital distance (https://aopwiki.org/events/1688)

Short Name: short male AGD

Key Event Component

Process	Object	Action
androgen receptor signaling pathway	Musculature of male perineum	disrupted

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	AdverseOutcome
Aop:306 - Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306)	AdverseOutcome
Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)	AdverseOutcome

Stressors

Name
Butylparaben
p,p'-DDE
Bis(2-ethylhexyl) phthalate
Dexamethasone
Fenitrothion
Finasteride
Flutamide
Ketoconazole
Linuron
Prochloraz
Procymidone

Name
Triticonazole
Vinclozolin
di-n-hexyl phthalate
Dicyclohexyl phthalate
butyl benzyl phthalate
monobenzyl phthalate
di-n-heptyl phthalate

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
perineum

Evidence for Perturbation by Stressor

Butylparaben

Butylparaben has been shown to cause decreased male AGD in rats following intrauterine exposure to 500 and 1000 mg/kg bw/day (Boberg et al, 2016; Zhang et al, 2014). A separate study using 600 mg/kg bw/day did not see an effect on male AGD (Boberg et al, 2008).

p,p'-DDE

p,p,DDE has been shown to cause decreased male AGD in rats following intrauterine exposure to 100-200 mg/kg bw/day (Loeffler & Peterson, 1999; Wolf et al, 1999).

Bis(2-ethylhexyl) phthalate

DEHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 300-1500 mg/kg bw/day (Christiansen et al, 2010; Gray et al, 2000; Howdeshell et al, 2007; Jarfelt et al, 2005; Kita et al, 2016; Li et al, 2013; Lin et al, 2009; Moore et al, 2001; Nardelli et al, 2017; Saillenfait et al, 2009; Wolf et al, 1999).

Dexamethasone

Dexamethasone has been shown to cause decreased male AGD in rats following intrauterine exposure to 0.1 mg/kg bw/day (Van den Driesche et al, 2012).

Fenitrothion

Fenitrothion has been shown to cause decreased male AGD in rats following intrauterine exposure to 25 mg/kg bw/day (Turner et al, 2002).

Finasteride

Finasteride has been shown to cause decreased male AGD in rats following intrauterine exposure to 100 mg/kg bw/day (Bowman et al, 2003).

Flutamide

Flutamide has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 16-100 mg/kg bw/day (Foster & Harris, 2005; Hass et al, 2007; Kita et al, 2016; McIntyre et al, 2001; Mylchreest et al, 1999; Scott et al, 2007; Welsh et al, 2007).

Ketoconazole

Ketoconazole has been shown to cause decreased male AGD in rats following intrauterine exposure to 50 mg/kg bw/day in one study (Taxvig et al, 2008), but no effect in another study using same dose (Wolf et al, 1999).

Linuron

Linuron has been shown to cause decreased male AGD in rats following intrauterine exposure to 50-100 mg/kg bw/day (Hotchkiss et al, 2004; McIntyre et al, 2002; Wolf et al, 1999).

Prochloraz

Prochloraz has been shown to cause decreased male AGD in rats following intrauterine exposure to 150-250 mg/kg bw/day (Laier et al, 2006; Noriega et al, 2005).

Procymidone

Procymidone has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 50-150 mg/kg bw/day (Hass et al, 2012; Hass et al, 2007; Wolf et al, 1999).

Triticonazole

Triticonazole has been shown to cause decreased male AGD in rats following intrauterine exposure to 150 and 450 mg/kg bw/day (Draskau et al, 2019).

Vinclozolin

Vinclozolin has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 50-200 mg/kg bw/day (Christiansen et al, 2009; Gray et al, 1994; Hass et al, 2007; Matsuura et al, 2005; Ostby et al, 1999; Schneider et al, 2011; Wolf et al, 2004).

di-n-hexyl phthalate

DnHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 500-750 mg/kg bw/day (Saillenfait et al, 2009a; Saillenfait et al, 2009b).

Dicyclohexyl phthalate

DCHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 350-750 mg/kg bw/day (Aydoğan Ahbab & Barlas, 2015; Hoshino et al, 2005; Saillenfait et al, 2009a).

butyl benzyl phthalate

BBP has been shown to cause decreased male AGD in rats following intrauterine exposure to 500-1000 mg/kg bw/day (Ema & Miyawaki, 2002; Gray et al, 2000; Hotchkiss et al, 2004; Nagao et al, 2000; Tyl et al, 2004).

monobenzyl phthalate

MBeP has been shown to cause decreased male AGD in rats following intrauterine exposure to 375 mg/kg bw/day (Ema et al, 2003).

di-n-heptyl phthalate

DHPP has been shown to cause decreased male AGD in rats following intrauterine exposure to 1000 mg/kg bw/day (Saillenfait et al, 2011).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	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)

Life Stage Applicability

Life Stage	Evidence
Foetal	High

Sex Applicability

Sex	Evidence
Male	High

A short AGD in male offspring is a marker of insufficient androgen action during critical fetal developmental stages (Schwartz et al, 2019; Welsh et al, 2008). A short AGD is thus a sign of undervirilization, which is also associated with a series of male reproductive disorders, including genital malformations and infertility in humans (Juul et al, 2014; Skakkebaek et al, 2001).

There are numerous human epidemiological studies showing associations with intrauterine exposure to anti-androgenic chemicals and short AGD in newborn boys alongside other reproductive disorders (Schwartz et al, 2019). This underscores the human relevance of this AO. However, in reproductive toxicity studies and chemical risk assessment, rodents (rats and mice) are what is tested on. The list of chemicals inducing short male AGD in male rat offspring is extensive, as evidenced by the 'stressor' list and reviewed by (Schwartz et al, 2019).

Key Event Description

The anogenital distance (AGD) refers to the distance between anus and the external genitalia. In rodents and humans, the male AGD is approximately twice the length as the female AGD (Salazar-Martinez et al, 2004; Schwartz et al, 2019). This sexual dimorphisms is a consequence of sex hormone-dependent development of secondary sexual characteristics (Schwartz et al, 2019). In males, it is believed that androgens (primarily DHT) activate AR-positive cells in non-myotic cells in the fetal perineum region to initiate differentiation of the perineal *levator ani* and *bulbocavernosus* (LABC) muscle complex (Ipulan et al, 2014). This AR-dependent process occurs within a critical window of development, around gestational days 15-18 in rats (MacLeod et al, 2010). In females, the absence of DHT prevents this masculinization effect from occurring.

The involvement of androgens in masculinization of the male fetus, including the perineum, has been known for a very long time (Jost, 1953), and AGD has historically been used to, for instance, sex newborn kittens. It is now well established that the AGD in newborns is a proxy readout for the intrauterine sex hormone milieu the fetus was developing. Too low androgen levels in XY fetuses makes the male AGD shorter, whereas excess (ectopic) androgen levels in XX fetuses makes the female AGD longer, in humans and rodents (Schwartz et al, 2019).

How it is Measured or Detected

The AGD is a morphometric measurement carried out by trained technicians (rodents) or medical staff (humans).

In rodent studies AGD is assessed as the distance between the genital papilla and the anus, and measured using a stereomicroscope with a micrometer eyepiece. The AGD index (AGDi) is often calculated by dividing AGD by the cube root of the body weight. It is important in statistical analysis to use litter as the statistical unit. This is done when more than one pup from each litter is examined. Statistical analyses is adjusted using litter as an independent, random and nested factor. AGD are analysed using body weight as covariate as recommended in Guidance Document 151 (OECD, 2013).

Regulatory Significance of the AO

In regulatory toxicology, the AGD is mandatory inclusions in OECD test guidelines used to test for developmental and reproductive toxicity of chemicals. Guidelines include 'TG 443 extended one-generation study', 'TG 421/422 reproductive toxicity screening studies' and 'TG 414 developmental toxicity study'.

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1880: 5α-reductase, inhibition leads to Decrease, DHT level (https://aopwiki.org/relationships/1880)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289)	**adjacent**	**High**	**High**
5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	**adjacent**	**High**	**High**

Relationship: 1935: Decrease, DHT level leads to Decrease, AR activation (https://aopwiki.org/relationships/1935)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288)	**adjacent**	**High**	**High**
Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)	**adjacent**	**High**	**Moderate**
5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	**adjacent**	**High**	**Moderate**

Relationship: 2128: Decrease, AR activation leads to decrease, transcription of genes by AR (https://aopwiki.org/relationships/2128)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	**adjacent**	**Moderate**	**Low**
Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

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)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human and other cells in culture	human and other cells in culture	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
During development and at adulthood	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Relationship: 2129: decrease, transcription of genes by AR leads to short male AGD (https://aopwiki.org/relationships/2129)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)	**adjacent**	**Moderate**	**Low**
Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306)	**adjacent**	**Moderate**	**Low**