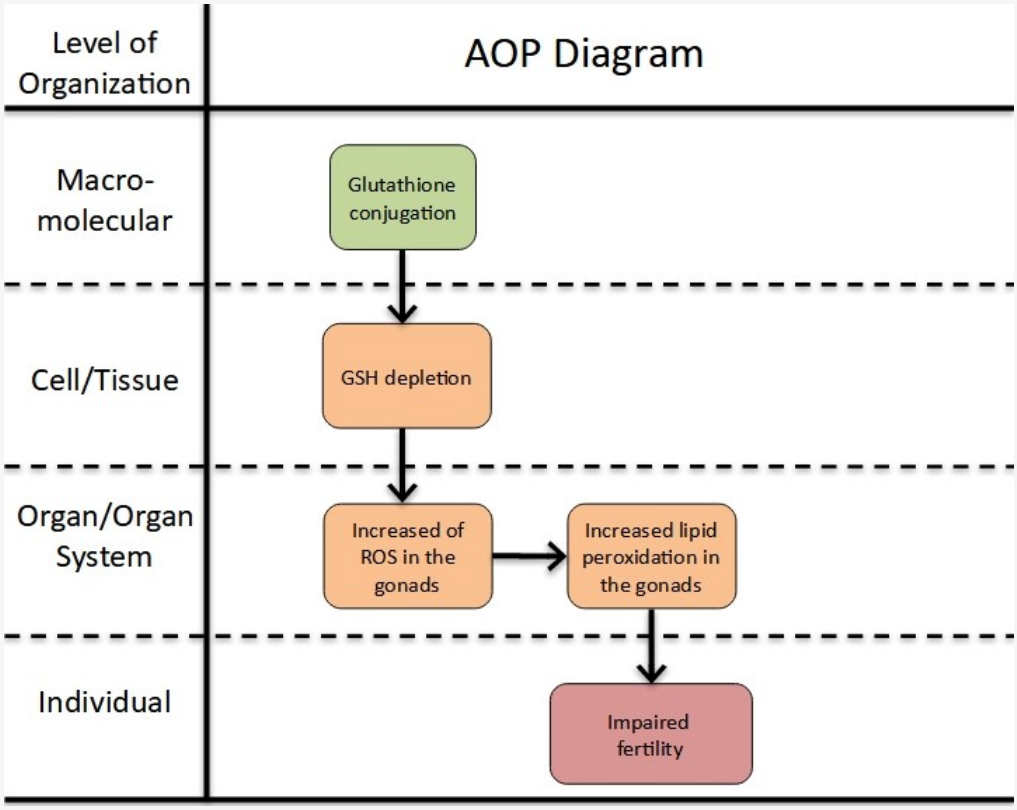
AOP ID and Title:

AOP 492: Glutathione conjugation leading to reproductive dysfunction via oxidative stress
Short Title: Glutathione conjugation leading to reproductive dysfunction

Graphical Representation



Authors

Status

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 Do not cite			

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
	MIE	2131	Conjugation, Glutathione	Conjugation, Glutathione
	KE	130	Depletion, GSH	Depletion, GSH
	KE	1115	Increased, Reactive oxygen species	Increased, Reactive oxygen species
	KE	1445	Increase, Lipid peroxidation	Increase, LPO
	AO	406	impaired, Fertility	impaired, Fertility

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Conjugation, Glutathione	adjacent	Depletion, GSH	High	High
Depletion, GSH	adjacent	Increased, Reactive oxygen species	High	High
Increased, Reactive oxygen species	adjacent	Increase, Lipid peroxidation	High	High
Increase, Lipid peroxidation	adjacent	impaired, Fertility	High	High

Overall Assessment of the AOP

References

Appendix 1

List of MIEs in this AOP

Event: 2131: Conjugation, Glutathione

Short Name: Conjugation, Glutathione

Key Event Component

Process	Object	Action
glutathione binding	glutathione conjugate	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

hepatocyte

Organ term

Organ term

liver

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Description

Glutathione, GSH (γ-L-glutamyl-L-cysteinyl-glycine) is a tripeptide synthesized in the intracellular media in a two-step process: bond between glutamic acid and cysteine by the enzyme glutamate-cystein ligase followed by the combination of the resulting dipeptide with a glycin, which is catalyzed by glutathione-synthetase (Lushchak 2012; Hellou, Ross, and Moon 2012; Aquilano, Baldelli, and Ciriolo 2014). In the oxidative stress pathway, GSH is used as substrate by different types and isoforms of enzymes, such as glutathione-reductases (GRs), glutathione-peroxidases (GPXs) and glutathione-transferases (GSTs).
Conjugation with glutathione might happen spontaneously, but it is a reaction primarily catalyzed by GSTs (X. Li 2009). This class of enzymes conjugates the tripeptide with toxic chemicals (e.g. arene, oxides, unsaturated carbonyls, organic halides) in order to neutralize them, making them harmless to cells through a Michael addition reaction (Forman, Zhang, and Rinna 2009; Lushchak 2012; Aquilano, Baldelli, and Ciriolo 2014). In this case, the sulfhydryl group acts as a nucleophile and binds, for instance, to an amine group or to an atom such as Cl, as well as attacks electrophilic sites of xenobiotics (X. Li 2009). Conjugates generated from this reaction, overall, are less toxic or are excreted from cells, which causes GSH depletion (Forman, Zhang, and Rinna 2009).

How it is Measured or Detected

Liquid chromatography–mass spectrometry (Pallante et al. 1986; Plakunov et al. 1987; Pflugmacher et al. 1998; Wiegand et al. 2001a; Dai et al. 2008; Dionisio, Gautam, and Fomsgaard 2019).

References

Lushchak, Volodymyr I. 2012. “Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions.” Journal of Amino Acids 2012 (February): 736837.

Hellou, Jocelyne, Neil W. Ross, and Thomas W. Moon. 2012. “Glutathione, Glutathione S-Transferase, and Glutathione Conjugates, Complementary Markers of Oxidative Stress in Aquatic Biota.” Environmental Science and Pollution Research International 19 (6): 2007–23.

Aquilano, Katia, Sara Baldelli, and Maria R. Ciriolo. 2014. “Glutathione: New Roles in Redox Signaling for an Old Antioxidant.” Frontiers in Pharmacology 5 (August): 196.

Forman, Henry Jay, Hongqiao Zhang, and Alessandra Rinna. 2009. “Glutathione: Overview of Its Protective Roles, Measurement, and Biosynthesis.” Molecular Aspects of Medicine 30 (1-2): 1–12.

Li, Xianchun. 2009. “Glutathione and Glutathione-S-Transferase in Detoxification Mechanisms.” In General, Applied and Systems Toxicology. Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470744307.gat166.

Pallante, S. L., C. A. Lisek, D. M. Dulik, and C. Fenselau. 1986. “Glutathione Conjugates. Immobilized Enzyme Synthesis and Characterization by Fast Atom Bombardment Mass Spectrometry.” Drug Metabolism and Disposition: The Biological Fate of Chemicals 14 (3): 313–18.

Plakunov, I., T. A. Smolarek, D. L. Fischer, J. C. Wiley Jr, and W. M. Baird. 1987. “Separation by Ion-Pair High-Performance Liquid Chromatography of the Glucuronide, Sulfate and Glutathione Conjugates Formed from Benzo[a]pyrene in Cell Cultures from Rodents, Fish and Humans.” Carcinogenesis 8 (1): 59–66.

Pflugmacher, S., C. Wiegand, A. Oberemm, K. A. Beattie, E. Krause, G. A. Codd, and C. E. Steinberg. 1998. “Identification of an Enzymatically Formed Glutathione Conjugate of the Cyanobacterial Hepatotoxin Microcystin-LR: The First Step of Detoxication.” Biochimica et Biophysica Acta 1425 (3): 527–33.

Wiegand, C., E. Krause, C. Steinberg, and S. Pflugmacher. 2001a. “Toxicokinetics of Atrazine in Embryos of the Zebrafish (Danio Rerio).” Ecotoxicology and Environmental Safety 49 (3): 199–205.

Dai, Ming, Ping Xie, Gaodao Liang, Jun Chen, and Hehua Lei. 2008. “Simultaneous Determination of Microcystin-LR and Its Glutathione Conjugate in Fish Tissues by Liquid Chromatography-Tandem Mass Spectrometry.” Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences 862 (1-2): 43–50.

Dionisio, Giuseppe, Maheswor Gautam, and Inge Sindbjerg Fomsgaard. 2019. “Identification of Azoxystrobin Glutathione Conjugate Metabolites in Maize Roots by LC-MS.” Molecules 24 (13). https://doi.org/10.3390/molecules24132473.

List of Key Events in the AOP

Event: 130: Depletion, GSH

Short Name: Depletion, GSH

Key Event Component

Process	Object	Action
abnormal glutathione level	glutathione	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
eukaryotic cell

Organ term

Organ term
liver

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Description

GSH depletion is commonly observed in different types of organs and cells (Deneke and Fanburg 1989; Lushchak 2012; Aquilano, Baldelli, and Ciriolo 2014). One of the main roles of this antioxidant is to sequester free radicals in order to prevent cell damage. A decline in GSH levels has been thoroughly related to the increase of reactive oxygen species, as well as to lipid peroxides, culminating in tissue oxidative stress (Comporti et al. 1991; Martin and Teismann 2009; Lushchak 2012; Aquilano, Baldelli, and Ciriolo 2014).

How it is Measured or Detected

- Photocolorimetric assays (Rahman 2007; Massarsky, Kozal, and Di Giulio 2017),
- HPLC (Afzal et al. 2002; J. Liu et al. 2010)
- Through commercial kits purchased from specialized companies.

References

Deneke, S. M., and B. L. Fanburg. 1989. “Regulation of Cellular Glutathione.” The American Journal of Physiology 257 (4 Pt 1): L163–73.

Lushchak, Volodymyr I. 2012. “Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions.” Journal of Amino Acids 2012 (February): 736837.

Aquilano, Katia, Sara Baldelli, and Maria R. Ciriolo. 2014. “Glutathione: New Roles in Redox Signaling for an Old Antioxidant.” Frontiers in Pharmacology 5 (August): 196.

Comporti, M., E. Maellaro, B. Del Bello, and A. F. Casini. 1991. “Glutathione Depletion: Its Effects on Other Antioxidant Systems and Hepatocellular Damage.” Xenobiotica; the Fate of Foreign Compounds in Biological Systems 21 (8): 1067–76.

Martin, Heather L., and Peter Teismann. 2009. “Glutathione--a Review on Its Role and Significance in Parkinson’s Disease.” FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 23 (10): 3263–72.

Rahman, Khalid. 2007. “Studies on Free Radicals, Antioxidants, and Co-Factors.” Clinical Interventions in Aging 2 (2): 219–36.

Massarsky, Andrey, Jordan S. Kozal, and Richard T. Di Giulio. 2017. “Glutathione and Zebrafish: Old Assays to Address a Current Issue.” Chemosphere 168 (February): 707–15.

Afzal, Mohammed, Aqeela Afzal, Andrew Jones, and Donald Armstrong. 2002. “A Rapid Method for the Quantification of GSH and GSSG in Biological Samples.” Methods in Molecular Biology 186: 117–22.

Liu, Jiaofang, Chunyan Bao, Xinhua Zhong, Chunchang Zhao, and Linyong Zhu. 2010. “Highly Selective Detection of Glutathione Using a Quantum-Dot-Based OFF–ON Fluorescent Probe.” Chemical Communications 46 (17): 2971–73

Event: 1115: Increased, Reactive oxygen species

Short Name: Increased, Reactive oxygen species

Key Event Component

Process	Object	Action
reactive oxygen species biosynthetic process	reactive oxygen species	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:186 - unknown MIE leading to renal failure and mortality	KeyEvent
Aop:213 - Inhibition of fatty acid beta oxidation leading to nonalcoholic steatohepatitis (NASH)	KeyEvent
Aop:303 - Frustrated phagocytosis-induced lung cancer	KeyEvent
Aop:383 - Inhibition of Angiotensin-converting enzyme 2 leading to liver fibrosis	KeyEvent
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:384 - Hyperactivation of ACE/Ang-II/AT1R axis leading to chronic kidney disease	KeyEvent
Aop:396 - Deposition of ionizing energy leads to population decline via impaired meiosis	KeyEvent
Aop:409 - Frustrated phagocytosis leads to malignant mesothelioma	KeyEvent
Aop:413 - Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	KeyEvent
Aop:416 - Aryl hydrocarbon receptor activation leading to lung cancer through IL-6 toxicity pathway	KeyEvent

AOP ID and Name	Event Type
Aop:418 - Aryl hydrocarbon receptor activation leading to impaired lung function through AHR-ARNT toxicity pathway	KeyEvent
Aop:386 - Deposition of ionizing energy leading to population decline via inhibition of photosynthesis	KeyEvent
Aop:387 - Deposition of ionising energy leading to population decline via mitochondrial dysfunction	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:451 - Interaction with lung resident cell membrane components leads to lung cancer	KeyEvent
Aop:476 - Adverse Outcome Pathways diagram related to PBDEs associated male reproductive toxicity	MolecularInitiatingEvent
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

ROS is a normal constituent found in all organisms.

Key Event Description

Biological State: increased reactive oxygen species (ROS)

Biological compartment: an entire cell -- may be cytosolic, may also enter organelles.

Reactive oxygen species (ROS) are O2- derived molecules that can be both free radicals (e.g. superoxide, hydroxyl, peroxyl, alcoxyl) and non-radicals (hypochlorous acid, ozone and singlet oxygen) (Bedard and Krause 2007; Ozcan and Ogun 2015). ROS production occurs naturally in all kinds of tissues inside various cellular compartments, such as mitochondria and peroxisomes (Drew and Leeuwenburgh 2002; Ozcan and Ogun 2015). Furthermore, these molecules have an important function in the regulation of several biological processes – they might act as antimicrobial agents or triggers of animal gamete activation and capacitation (Goud et al. 2008; Parrish 2010; Bisht et al. 2017).
However, in environmental stress situations (exposure to radiation, chemicals, high temperatures) these molecules have its levels drastically increased, and overly interact with macromolecules, namely nucleic acids, proteins, carbohydrates and lipids, causing cell and tissue damage (Brieger et al. 2012; Ozcan and Ogun 2015).

How it is Measured or Detected

Photocolorimetric assays (Sharma et al. 2017; Griendling et al. 2016) or through commercial kits purchased from specialized companies.

References

Bedard, Karen, and Karl-Heinz Krause. 2007. “The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology.” Physiological Reviews 87 (1): 245–313.

Ozcan, Ayla, and Metin Ogun. 2015. “Biochemistry of Reactive Oxygen and Nitrogen Species.” In Basic Principles and Clinical Significance of Oxidative Stress, edited by Sivakumar Joghi Thatha Gowder. Rijeka: IntechOpen.

Drew, Barry, and Christiaan Leeuwenburgh. 2002. “Aging and the Role of Reactive Nitrogen Species.” Annals of the New York Academy of Sciences 959 (April): 66–81.

Goud, Anuradha P., Pravin T. Goud, Michael P. Diamond, Bernard Gonik, and Husam M. Abu-Soud. 2008. “Reactive Oxygen Species and Oocyte Aging: Role of Superoxide, Hydrogen Peroxide, and Hypochlorous Acid.” Free Radical Biology & Medicine 44 (7): 1295–1304.

Parrish, A. R. 2010. “2.27 - Hypoxia/Ischemia Signaling.” In Comprehensive Toxicology (Second Edition), edited by Charlene A. McQueen, 529–42. Oxford: Elsevier.

Bisht, Shilpa, Muneeb Faiq, Madhuri Tolahunase, and Rima Dada. 2017. “Oxidative Stress and Male Infertility.” Nature Reviews. Urology 14 (8): 470–85.

Brieger, K., S. Schiavone, F. J. Miller Jr, and K-H Krause. 2012. “Reactive Oxygen Species: From Health to Disease.” Swiss Medical Weekly 142 (August): w13659.

Sharma, Gunjan, Nishant Kumar Rana, Priya Singh, Pradeep Dubey, Daya Shankar Pandey, and Biplob Koch. 2017. “p53 Dependent Apoptosis and Cell Cycle Delay Induced by Heteroleptic Complexes in Human Cervical Cancer Cells.” Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie 88 (April): 218–31.

Griendling, Kathy K., Rhian M. Touyz, Jay L. Zweier, Sergey Dikalov, William Chilian, Yeong-Renn Chen, David G. Harrison, Aruni Bhatnagar, and American Heart Association Council on Basic Cardiovascular Sciences. 2016. “Measurement of Reactive Oxygen Species, Reactive Nitrogen Species, and Redox-Dependent Signaling in the Cardiovascular System: A Scientific Statement From the American Heart Association.” Circulation Research 119 (5): e39–75.

Event: 1445: Increase, Lipid peroxidation

Short Name: Increase, LPO

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:329 - Excessive reactive oxygen species production leading to mortality (3)	KeyEvent
Aop:413 - Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	KeyEvent
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
fish	fish	Moderate	NCBI

ROS is a normal constituent found in all organisms, therefore, all organisms containing lipid membranes may be affected by lipid peroxidation.

Structure: Regardless of sex or life stage, when exposed to free radicals, there is potential for lipid peroxidation as a auxiliary response where there are lipid membranes.

Key Event Description

Lipid peroxidation is the direct damage to lipids in the membrane of the cell or the membranes of the organelles inside the cells. Ultimately the membranes will break due to the build-up damage in the lipids. This is mainly caused by oxidants which attack lipids specifically, since these contain carbon-carbon double bonds. During lipid peroxidation several lipid radicals are formed in a chain reaction. These reactions can interfere and stimulate each other. Antioxidants, such as vitamin E, can react with lipid peroxy radicals to prevent further damage in the cell (Cooley et al. 2000).

How it is Measured or Detected

The main product of lipid peroxidation, malondialdehyde and 4-hydroxyalkenals, is used to measure the degree of this process. This is measured by photocolorimetric assays, quantification of fatty acids by gaseous liquid chromatography (GLC) or high performance (HPLC) (L. Li et al. 2019; Jin et al. 2010a) or through commercial kits purchased from specialized companies.

References

Cooley HM, Evans RE, Klaverkamp JF. 2000. Toxicology of dietary uranium in lake whitefish (Coregonus clupeaformis). Aquatic Toxicology. 48(4):495–515. https://doi.org/10.1016/S0166-445X(99)00057-0

Jin, Yuanxiang, Xiangxiang Zhang, Linjun Shu, Lifang Chen, Liwei Sun, Haifeng Qian, Weiping Liu, and Zhengwei Fu. 2010a. “Oxidative Stress Response and Gene Expression with Atrazine Exposure in Adult Female Zebrafish (Danio Rerio).” Chemosphere 78 (7): 846–52.

Li, Luxiao, Shanshan Zhong, Xia Shen, Qiujing Li, Wenxin Xu, Yongzhen Tao, and Huiyong Yin. 2019. “Recent Development on Liquid Chromatography-Mass Spectrometry Analysis of Oxidized Lipids.” Free Radical Biology & Medicine 144 (November): 16–34.

List of Adverse Outcomes in this AOP

Event: 406: impaired, Fertility

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	AdverseOutcome
Aop:51 - PPARα activation leading to impaired fertility in adult male rodents	AdverseOutcome
Aop:18 - PPARα activation in utero leading to impaired fertility in males	AdverseOutcome
Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility	AdverseOutcome
Aop:348 - Inhibition of 11β-Hydroxysteroid Dehydrogenase leading to decreased population trajectory	KeyEvent
Aop:349 - Inhibition of 11β-hydroxylase leading to decresed population trajectory	KeyEvent
Aop:396 - Deposition of ionizing energy leads to population decline via impaired meiosis	KeyEvent
Aop:398 - Inhibition of ALDH1A (RALDH) leading to impaired fertility via disrupted meiotic initiation of fetal oogonia of the ovary	AdverseOutcome
Aop:492 - Glutathione conjugation leading to reproductive dysfunction via oxidative stress	AdverseOutcome

Biological Context

Level of Biological Organization

Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Terms	Evidence	Links
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	High

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: 2877: Conjugation, Glutathione leads to Depletion, GSH

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glutathione conjugation leading to reproductive dysfunction via oxidative stress	adjacent	High	High

Relationship: 2878: Depletion, GSH leads to Increased, Reactive oxygen species

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glutathione conjugation leading to reproductive dysfunction via oxidative stress	adjacent	High	High

Relationship: 2460: Increased, Reactive oxygen species leads to Increase, LPO

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Oxidation and antagonism of reduced glutathione leading to mortality via acute renal failure	adjacent	High	Moderate
Glutathione conjugation leading to reproductive dysfunction via oxidative stress	adjacent	High	High

Relationship: 2879: Increase, LPO leads to impaired, Fertility

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glutathione conjugation leading to reproductive dysfunction via oxidative stress	adjacent	High	High