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Adverse outcome pathway: Framework, application, and challenges in chemical risk assessment

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The number of chemicals that require risk assessment evaluation has been rising worldwide. To protect human health and the environment, a large number of commercially available substances require risk evaluation. Traditionally, the assessment of the health risks of chemical exposure has relied mostly on directly measured adverse outcomes (*e.g.*, death, reproduction, growth, development dysfunction) by applying *in vivo* toxicity tests. These traditional approaches for risk assessment of chemical compounds are time-consuming, expensive, low-throughput, and yield little information on mechanistic toxicity (Ankley et al., 2010; Hutchinson et al., 2013; Nie et al., 2014; Zhu et al., 2013; Wang et al., 2015). Moreover, it is debatable whether extrapolations can be made from one species to another, as well as whether high-dose animal studies can predict risks to humans from much lower-dose exposures (Adeleye et al., 2015). Furthermore, using large numbers of animals raises ethical issues, and traditional resource-intensive standard *in vivo* toxicology studies are not feasible for the regulatory testing of all chemicals requiring health or environmental risk evaluation (Ankley et al., 2010). Thus, there is an urgent and growing need for more efficient, cost-effective methods to decrease the need for animals in the assessment of the hazards and risks of chemicals.

In 2007, the US National Research Council (NRC) published the report Toxicity Testing in the 21st Century: A Vision and a Strategy (NRC, 2007). The essence of this report was a call to transform toxicity testing from a system based on high-dose studies in laboratory animals to one based primarily on *in vitro* methods that evaluate changes in normal cellular signaling pathways using cells or tissues relevant to humans. The term "toxicity pathway" was applied to test whether a normal signaling process was significantly perturbed, and whether the result would be an adverse cellular outcome (NRC, 2007; Adeleye et al., 2015). Recently, a mechanistic pathway-based concept has been advocated for chemical risk assessment, named the adverse outcome pathway (AOP) (Ankley et al., 2010). An AOP framework starts with chemical reactions during the molecular initiating event(s), which leads to a series of further events (*e.g.*, key events), and finally ends up with a specific adverse outcome (*e.g.*, death, disease, reproductive failure, or developmental dysfunction). In this context, the AOP has been proposed as a framework to describe the causal linkage between the initial molecular events and an adverse outcome across levels of biological organization (*e.g.*, cell, tissue, organ, individual, and population), leading to an adverse outcome that is relevant to risk assessment and regulatory decision-making (Ankley et al., 2010; Groh et al., 2015). The concept of AOP differs from the concept of "toxicity pathways", which focuses on the molecular initiating event and on key measurable endpoints, but not on the adverse outcome. AOP represents the linkage between the molecular initiating event and the following progression of a defined series of key events that are measurable changes in biological state and necessary for an adverse outcome to occur (Ankley et al., 2010; Landesmann et al., 2013). Hence, AOP includes the whole process of the molecular initiating event and adverse consequences. Based on well-defined measurable molecular initiating events and key events of certain chemicals induced by *in vitro* or *in vivo* assays, AOPs could be used to evaluate the potential adverse outcomes of certain new chemicals (Phillips et al., 2015). Lee et al. (2015) summarized the utility of AOPs for biomarker-based environmental risk assessment and examined the use of AOPs in environmental risk assessment using aquatic organisms, such as fish and aquatic invertebrates (Fig. 1). Further studies regarding the concept, development, application, and challenges of AOP have been published (Vinken et al., 2013; Vinken, 2013; Adeleye et al., 2015; Villeneuve et al., 2014a,2014b; Hutchinson et al., 2013; Tollefsen et al., 2014; Patlewicz et al., 2015; Groh et al., 2015; Becker et al., 2015; Knapen et al., 2015).

For example, a recent paper entitled "Inhibition of spawning in zebrafish (*Danio rerio*): Adverse outcome pathways of ethinylestradiol" (Cosme et al., 2015) is a case study showing that exposure to an estrogenic compound can lead to an adverse outcome such as inhibition of spawning in

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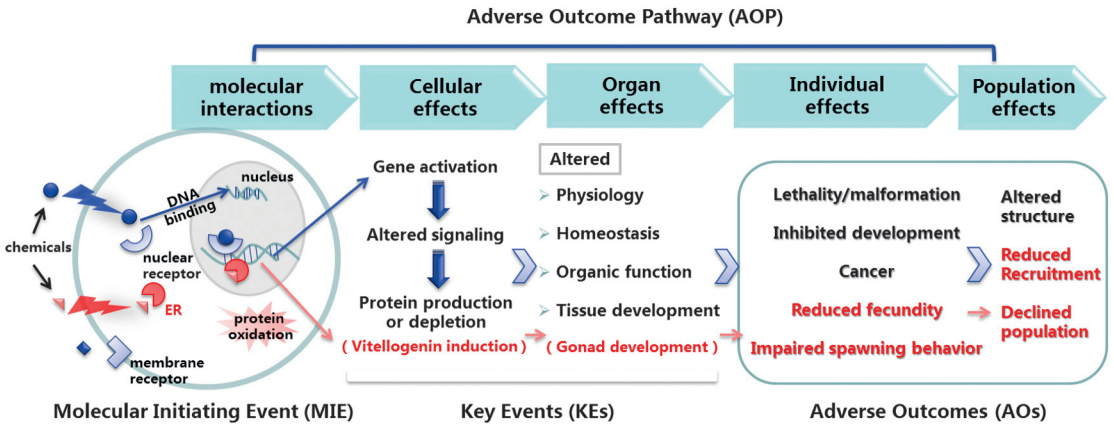

Fig. 1 – Flow diagram depicting an Adverse Outcome Pathway (AOP), representing common chemicals triggering molecular initiating events leading to a sequential series of higher order effects to produce an adverse outcome. The red branching is a typical example indicating chemicals *via* an estrogen receptor (ER) pathway leading to an adverse outcome. This diagram was modified from Ankley et al. (2010), Vinken et al. (2013), Tollefsen et al. (2014), and Lee et al. (2015).

zebrafish. The authors examined the effects of the estrogen receptor agonist ethinylestradiol (EE2) on the pathways controlling follicular development, steroidogenesis, oocyte maturation, ovulation, and spawning success in adult zebrafish. This is a typical AOP that includes the molecular initiating event of binding to the estrogenic receptor (ER) and altered reproduction (*e.g.*, decrease spawning or fecundity) at the whole-organism level, with consequent declining effects on population. In this AOP framework, intermediate levels of biological organization include decreased vitellogenin (VTG) mRNA expression and protein synthesis, followed by decreased circulatory levels of VTG, leading to less uptake of VTG into oocytes and then impairment of oocyte development in the ovary. The study demonstrates the significance of defining the impacts of the toxicant at the molecular/cellular, organ, and individual level and how connections among these impacts can be used to describe the AOPs that mediate the action of the toxicant (Cosme et al., 2015). The results suggest that the AOP framework is effective at providing a detailed description of a toxicant's adverse effects on an organism and has the potential to be utilized to evaluate the hazards posed by a toxicant.

The AOP framework forms a basis for the development of new non-animal test methods (Ankley et al., 2010). As fish embryos (pre-hatch stages) and eleuthereoembryos – the time period between immediately post-hatching and independent feeding – are non-protected life-stages, these early life-stages are considered as alternative testing models in the European Union and the United States (Belanger et al., 2010; Scholz et al., 2008). In addition, in contrast to cell-based *in vitro* assays, fish embryos provide the complexity and interactions of an intact organism, enabling the evaluation of adverse chemical effects on multiple target organs and developmental stages during embryogenesis (Yozzo et al., 2013). The zebrafish embryos have rapid embryogenesis, and they are small in size and transparent; they are also easy to maintain and handle, and to be genetically manipulated during embryogenesis. Zebrafish embryos have become a preferred model for rapid and high-throughput screening of compounds for developmental toxicity and mechanisms of toxicant exposure (Lammer et al., 2009; Scholz and Mayer, 2008). These advantages make zebrafish embryos a good model for developing AOPs. For instance, a recent study reported that exposure of zebrafish embryos to the goitrogens phenylthiourea and methimazole induced adverse outcomes such as reduction of locomotion and craniofacial malformation. These adverse outcomes can be linked to the molecular initiating event, the altered expression of gene coding for enzymes involved in thyroid hormone (TH) synthesis, and to a key event, reduced thyroxin levels (Fetter et al., 2015). THs are particularly important in fetal development and growth; they act by binding to specific thyroid receptors (TRs), and play an important role during early embryogenesis and larval development in fish. Endocrine disrupting chemicals (EDCs) can have a direct impact on TH synthesis, transport, and binding, and can also interfere with the functioning of the thyroid system by inducing feedback mechanisms triggered by changes in the concentration of circulating THs (Kloas and Lutz, 2006). A previous study suggested that zebrafish embryos/larvae represent a valuable and powerful alternative for novel studies of large-scale screening of chemicals that can disrupt the activity of thyroid hormones (Yu et al., 2010). Therefore, developing embryos/larvae show great promise for the development of AOPs for risk assessment of chemicals that have thyroid endocrine disruption.

Although AOPs hold great promise as useful tools for predicting adverse outcomes of chemicals in risk assessments by measurement of pathway- and mechanistic-based events, the use of AOPs in a full risk assessment may still face challenges. For instance, many molecular events induced by chemicals are dose-dependent, while interspecies differences regarding sensitivity to chemicals are another big challenge for the development of AOPs. Furthermore, the AOPs should also account for the many environmental factors that could modify molecular events as well as affect the adverse outcomes, when using laboratory data to predict them in the field. Nevertheless, the AOP framework approach offers great potential for improvement of traditional toxicity testing by measuring targeted endpoints, including assay specificity

relative to the molecular events, or mechanisms of action and related adverse outcomes. As the AOP conceptual framework is improved by many researchers, the AOPs will be a powerful approach to address risk assessment needs.

REFERENCES

Adeleye, Y., Andersen, M., Clewell, R., Davies, M., Dent, M., Edwards, S., et al., 2015. Implementing toxicity testing in the 21st century (TT21C): making safety decisions using toxicity pathways, and progress in a prototype risk assessment. Toxicology 332, 102–111.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., et al., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741.

Becker, R.A., Ankley, G.T., Edwards, S.W., Kennedy, S.W., Linkov, I., Meek, B., et al., 2015. Increasing scientific confidence in adverse outcome pathways: application of tailored Bradford-Hill considerations for evaluating weight of evidence. Regul. Toxicol. Pharmacol. 72, 514–537.

Belanger, S., Balon, E., Rawlings, J.M., 2010. Saltatory ontogeny of fishes and sensitive early life stages for ecotoxicology tests. Aquat. Toxicol. 97, 88–95.

Cosme, M.M., Lister, A.L., Van Der Kraak, G., 2015. Inhibition of spawning in zebrafish (*Danio rerio*): adverse outcome pathways of quinacrine and ethinylestradiol. Gen. Comp. Endocrinol. 219, 89–101.

Fetter, E., Baldauf, L., Fonte, D.F.D., Ortmann, J., Scholz, S., 2015. Comparative analysis of goitrogenic effects of phenylthiourea and methimazole in zebrafish embryos. Reprod. Toxicol. (in press).

Groh, K.J., Carvalho, R.N., Chipman, J.K., Denslow, N.D., Halder, M., Murphy, C.A., et al., 2015. Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology. Chemosphere 120, 764–777.

Hutchinson, T.H., Lyons, B.P., Thain, J.E., Law, R.J., 2013. Evaluating legacy contaminants and emerging chemicals in marine environments using adverse outcome pathways and biological effects-directed analysis. Mar. Pollut. Bull. 74, 517–525.

Kloas, W., Lutz, I., 2006. Amphibians as model to study endocrine disrupters. J. Chromatogr. A 1130, 16–27.

Knapen, D., Vergauwen, L., Villeneuve, D.L., Ankley, G.T., 2015. The potential of AOP networks for reproductive and developmental toxicity assay development. Reprod. Toxicol. 56, 52–55.

Lammer, E., Carr, G.J., Wendler, K., Rawlings, J.M., Belanger, S.E., Braunbeck, T., 2009. Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? Comp. Biochem. Physiol. C 149, 196–209.

Landesmann, B., Mennecozzi, M., Berggren, E., Whelan, M., 2013. Adverse outcome pathway-based screening strategies for an animal-free safety assessment of chemicals. Altern. Lab. Anim. 41, 461–471.

Lee, J.W., Won, E.J., Raisuddin, S., Lee, J.S., 2015. Significance of adverse outcome pathways in biomarker-based environmental risk assessment in aquatic organisms. J. Environ. Sci. 35, 115–127.

Nie, J., Shi, J., Duan, X.L., Wang, B.B., Huang, N., Zhao, X.G., 2014. Health risk assessment of dietary exposure to polycyclic aromatic hydrocarbons in Taiyuan. Chin. J. Environ. Sci. 26, 432–439.

NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies Press, Washington, DC.

Patlewicz, G., Simon, T.W., Rowlands, J.C., Budinsky, R.A., Becker, R.A., 2015. Proposing a scientific confidence framework to help support the application of adverse outcome pathways for regulatory purposes. Regul. Toxicol. Pharmacol. 71, 463–477.

Phillips, M.B., Leonard, J.A., Grulke, C.M., Chang, D.T., Edwards, S.W., Brooks, R., et al., 2015. A workflow to investigate exposure and pharmacokinetic influences on high-throughput in vitro chemical screening based on adverse outcome pathways. Environ. Health Perspect. (in press).

Scholz, S., Mayer, I., 2008. Molecular biomarkers of endocrine disruption in small model fish. Mol. Cell. Endocrinol. 293, 57–70.

Scholz, S., Fischer, S., Gundel, U., Kuster, E., Luckenbach, T., Voelker, D., 2008. The zebrafish embryo model in environmental risk assessment—applications beyond acute toxicity testing. Environ. Sci. Pollut. Res. 15, 394–404.

Tollefsen, K.E., Scholz, S., Cronin, M.T., Edwards, S.W., de Knecht, J., Crofton, K., et al., 2014. Applying adverse outcome pathways (AOPs) to support integrated approaches to testing and assessment (IATA). Regul. Toxicol. Pharmacol. 70, 629–640.

Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., et al., 2014a. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci. 142, 312–320.

Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., et al., 2014b. Adverse outcome pathway development II: best practices. Toxicol. Sci. 142, 321–330.

Vinken, M., 2013. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology 312, 158–165.

Vinken, M., Landesmann, B., Goumenou, M., Vinken, S., Shah, I., Jaeschke, H., et al., 2013. Development of an adverse outcome pathway from drug-mediated bile salt export pump inhibition to cholestatic liver injury. Toxicol. Sci. 136, 97–106.

Wang, X.F., Huang, P., Liu, Y., Du, H., Wang, X.N., Wang, M.M., et al., 2015. Role of nitric oxide in the genotoxic response to chronic microcystin-LR exposure in human–hamster hybrid cells. J. Environ. Sci. 29, 201–218.

Yozzo, K.L., McGee, S.P., Volz, D.C., 2013. Adverse outcome pathways during zebrafish embryogenesis: a case study with paraoxon. Aquat. Toxicol. 126, 346–354.

Yu, L., Deng, J., Shi, X., Liu, C., Yu, K., Zhou, B., 2010. Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic–pituitary–thyroid axis of zebrafish larvae. Aquat. Toxicol. 97, 226–233.

Zhu, N., Li, H.Y., Li, G.K., Sang, N., 2013. Coking wastewater increases micronucleus frequency in mouse *in vivo* via oxidative stress. J. Environ. Sci. 25, 2123–2129.