BIOACCUMULATION

Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from, an organism.[1] The OECD test guidelines below can be used to assess a substance's potential to bioaccumulate.

METHOD	TEST PRINCIPLE	APPLICABILITY DOMAIN	DATA INTERPRETATION
OECD TG 319A: Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)[2] OECD TG 319B: Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)[3] Adopted in 2018	*In vitro* intrinsic clearance (i.e. biotransformation rate) in primary hepatocytes or liver S9 subcellular fractions from rainbow trout. Although these assays use primary cells and S9, respectively, from fish killed for these experiments, the number of fish used is significantly reduced compared to the number of live fish used in OECD TG 305 on bioaccumulation in fish: aqueous and dietary exposure, and live fish are not exposed to the test chemical.	To date, more than 250 unique chemicals have been tested in the TG 319A/B assays, including a broad range of chemical classes and covering a wide range of hydrophobicity. OECD TGs 319A/B are applicable to ionisable organic compounds.[4-5] When testing five substances with different physicochemical characteristics using TG 319A, extrapolated bioconcentration factors correlated with K_{ow}, and there was no distinct tendency toward over- or underprediction of bioconcentration factors (BCFs) compared to *in vivo* fish values."[6]	Liver intrinsic clearance is estimated from the determined *in vitro* intrinsic clearance and may be used directly as an input to physiologically-based toxicokinetic (PBTK) models for fish bioaccumulation assessment.[7-8] Alternatively, this value may be extrapolated to a whole-body (*in vivo*) biotransformation rate constant using an appropriate *in vitro* to *in vivo* extrapolation (IVIVE) model. An IVIVE model applicable to rainbow trout was described by Nichols et al.[9] and is included as one example in the OECD Guidance Document on RT-HEP and RT-S9.[10] The "incorporation of experimentally derived metabolism rates leads to more realistic BCF estimations" than *in silico* predictions alone.[6]

The OECD case study on the use of Integrated Approaches for Testing and Assessment (IATA) for bioaccumulation provides a weight-of-evidence framework for bioaccumulation.[4] Multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common metrics; namely the BCF, bioaccumulation factor (BAF), biomagnification factor (BMF) and trophic magnification factor (TMF). These LoE can comprise *in silico* predictions, combinations of *in vitro* and *in silico* data, e.g., *in vitro-in vivo* extrapolation (IVIVE), and measured laboratory or field data.[4]

Note: Primary fish cells and S9 are used in TG 319A and 319B, respectively. However, some regulatory agencies may require reduction and refinement approaches while complete replacement methods are undergoing development and validation.

References

1. OECD. Case study on the use of Integrated Approaches for Testing and Assessment (IATA) for bioaccumulation - ninth review cycle. OECD Series on Testing and Assessment No. 404. 2023. https://doi.org/10.1787/8bc8ad6f-en.
2. OECD. Test No. 319A: Determination of *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP). 2018. https://doi.org/10.1787/9789264303218-en.
3. OECD. Test No. 319B: Determination of *in vitro* intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9). 2018. https://doi.org/10.1787/9789264303232-en.
4. Chen Y, Hermens JL, Jonker MT, et al. Which molecular features affect the intrinsic hepatic clearance rate of ionizable organic chemicals in fish? *Environ Sci Technol*. 2016;50(23):12722-12731. https://doi.org/10.1021/acs.est.6b03504
5. Droge STJ, Scherpenisse P, Arnot JA, et al. Screening the baseline fish bioconcentration factor of various types of surfactants using phospholipid binding data. *Environ Sci Process Impacts*. 2021;23(12):1930-1948. https://doi.org/10.1039/d1em00327e
6. Kosfeld V, Fu Q, Ebersbach I, et al. Comparison of alternative methods for bioaccumulation assessment: scope and limitations of in vitro depletion assays with rainbow trout and bioconcentration tests in the freshwater amphipod hyalella azteca. *Environ Toxicol Chem*. 2020;39(9):1813-1825. https://doi.org/10.1002/etc.4791.
7. Stadnicka-Michalak J, Tanneberger K, Schirmer K, et al. Measured and modeled toxicokinetics in cultured fish cells and application to in vitro-in vivo toxicity extrapolation. *PLoS One*. 2014;9(3):e92303. https://doi.org/10.1371/journal.pone.0092303
8. Brinkmann M, Schlechtriem C, Reininghaus M, et al. Cross-species extrapolation of uptake and disposition of neutral organic chemicals in fish using a multispecies physiologically-based toxicokinetic model framework. *Environ Sci Technol*. 2016;50(4):1914-23. https://doi.org/10.1021/acs.est.5b06158
9. Nichols JW, Huggett DB, Arnot JA, et al. Toward improved models for predicting bioconcentration of well-metabolized compounds by rainbow trout using measured rates of in vitro intrinsic clearance. *Environ Toxicol Chem*. 2013;32(7):1611-22. https://doi.org/10.1002/etc.2219
10. OECD. Guidance Document on the determination of in vitro intrinsic clearance using cryopreserved hepatocytes (RT-HEP) or liver S9 sub-cellular fractions (RT-S9) from rainbow trout and extrapolation to in vivo intrinsic clearance. OECD Series on Testing and Assessment No. 280. 2018. https://one.oecd.org/document/ENV/JM/MONO(2018)12/en/pdf.